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Increased Plasma Levels of FABP4 and PTEN are Associated with More Severe Insulin Resistance in Women with Gestational Diabetes Mellitus

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: The aim of this study was to investigate the relationship between plasma fatty acid binding protein 4 (FABP4), phosphatase and tensin homolog (PTEN), and insulin resistance in patients with gestational diabetes mellitus (GDM).

Material/Methods: Plasma FABP4 and PTEN were determined by ELISA in GDM patients (GDM group, n=30) and in euglycemic pregnant women (control group, n=30). The clinical features, body mass index (BMI), homeostasis model assessment of insulin resistance (HOMA-IR), and lipid profiles were compared between the 2 groups. The influence of risk factors on insulin resistance, including BMI, lipid profiles, FABP4, and PTEN, were further investigated by multiple-factor stepwise regression analysis.

Results: Higher levels of BMI, ΔBMI, triglyceride (TG), fasting plasma glucose (FPG), 2-hour plasma glucose (2hPG), fasting insulin, HOMA-IR, FABP4, PTEN, and lower level of high-density lipoprotein cholesterol (HDL-C) were found in the GDM patients than in the controls (all $P<0.005$). The plasma FABP4 was 1.47±0.25 *vs.* 0.20±0.07 ng/ml in the GDM and control group, respectively ($P<0.0001$). Plasma PTEN was 6.46±1.57 *vs.* 4.72±0.82 ng/ml in the GDM and control group, respectively ($P<0.0001$). There was a positive relation between plasma FABP4 and PTEN when all blood samples, including GDM and control groups, were analyzed ($P<0.05$). The multiple-factor regression analysis revealed that plasma FABP4, TG, and PTEN were independent risk factors for increased insulin resistance.

Conclusions: GDM patients have more severe insulin resistance compared to euglycemic pregnant women. Higher levels of plasma FABP4 and PTEN are associated with increased insulin resistance and may participate in the pathogenesis of insulin resistance during gestation.

MeSH Keywords: Diabetes, Gestational • Fatty Acid-Binding Proteins • Insulin Resistance • PTEN Phosphohydrolase

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Background

Gestational diabetes mellitus (GDM) may give rise to higher risks of multiple complications for mother and fetus, including miscarriage, preterm delivery, pregnancy hypertension, embryonic abnormalities, macrosomia, and even fetal death [1,2]. Prevalence of GDM in China is increasing, from 3.7% in 1995 [3] to 5.1% in 2008 [4].

Increased insulin resistance is one of the main mechanisms for the development of GDM. Previous studies indicate that there are 2 types of insulin resistance in GDM. One is called as "physiological insulin resistance", which is mediated by pregnancy-related hormones and will aggravate during the third trimester. The other is called "chronic insulin resistance", which already existed before pregnancy [5]. Insulin sensitivity in pregnant women will gradually decrease as the gestational weeks increase. Inadequate compensatory secretion of insulin can result in gestational diabetes mellitus. Hyperglycemia will reverse to normal after delivery for most GDM patients. However, in some patients it may extend to postpartum. Near half of GDM patients will develop to type 2 diabetes and obesity in the future [6]. Furthermore, the risk of type 2 diabetes in their offspring is also increased [7–9].

Adiposity fatty acid-binding protein 4 (FABP4) is a member of the lipid-binding protein super-family. As an important intracellular fatty acid carrier protein, it is widely involved in fatty acid uptake, transport, and metabolism. The roles of FABP4 in the development of metabolic syndrome, type 2 diabetes mellitus, cardiovascular disease [10,11], preeclampsia and GDM [12] are of great concern.

Phosphatase and tensin homolog (PTEN) was first discovered as a tumor suppressor gene. Deletion or inactivation of this gene can lead to a variety of cancers. Recent findings indicate that it plays a role in the regulation of the insulin signal transduction pathway. The higher expression of this gene may contribute to more severe insulin resistance. A complex of FABP4 and PTEN was recently detected and is presumed to play a role in the pathogenesis of insulin resistance [13].

Therefore, we hypothesized that increased levels of plasma FABP4 and PTEN are associated with GDM. The relationship between FABP4, PTEN, HOMA-IR, and other biochemical parameters in GDM were further investigated.

Material and Methods

Participants

A total of 30 pregnant women with GDM (GDM group) and 30 euglycemic pregnant women (control group) were recruited. The mean age of the GDM patients and the control group was 31.83±3.91 and 26.53±1.91 years (P>0.05). Patients with renal diseases, preeclampsia, or systemic inflammatory diseases were excluded. The study protocol was approved by Inner Mongolia Medical University ethics committee. The written informed consents were obtained from all participants.

Definition of our research

GDM was diagnosed if 1 or more plasma glucose levels were elevated during an oral glucose tolerance test with 75 g glucose, according to the criteria of Standards of Medical Care in Diabetes 2011 [14]. The following plasma glucose threshold was used: fasting blood glucose ≥5.1 mmol/l; 1-h blood glucose ≥10.0 mmol/l; 2-h blood glucose ≥8.5 mmol/l.

BMI was calculated as weight divided by squared height before pregnancy. ΔBMI is the net increment of BMI during pregnancy. HOMA-IR was calculated as previously described: HOMA-IR=(FPG × FINS)/22.5 (FINS, fasting insulin) [7].

Biochemistry and hormonal assays

Maternal blood samples were collected at 24–28 weeks of gestation. Blood samples were taken after an overnight fast. At the time of blood sampling, none of the women were in labor. Serum insulin was determined by 2-site chemiluminescent enzyme immunometric assay in an Immulite automated analyzer (DPC, Siemens, Marburg, Germany). FABP4 and PTEN were measured by ELISA according to the manufacturers' instructions (R&D Systems China, Shanghai). TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), FPG, and 2-hPG were measured by standard laboratory methods in a certificated laboratory.

Statistical analysis

SPSS software version 19.0 was used for statistical analysis. The differences between the GDM and control groups were assessed by t test. Correlations were conducted by using the Pearson correlation analysis method. To evaluate the effects of covariates on insulin resistance, multivariate linear regression analyses were undertaken. Before performing multivariate analyses, non-normally distributed parameters were logarithmically transformed. A P-value <0.05 was considered as statistically significant.

Results

The GDM group had higher levels of BMI, ΔBMI, TG, FPG, 2-hPG, FINS, HOMA-IR, FABP4, and PTEN than the control group (Table 1). Serum HDL-C was lower in the GDM group compared

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Table 1. Baseline characteristics of the study population.

	GDM N=30	Controls N=30	t	*P*
Age	31.83±3.91	26.53±1.91	6.68	>0.05
Body Mass Index (Kg/m^2)	21.80±1.02	19.18±0.68	11.663	<0.0001*
ΔBMI (Kg/m^2)	7.38±1.14	6.19±0.91	4.4439	<0.0001*
FPG (mmol/L)	5.53±0.64	4.18±0.45	9.49	<0.0001*
2hPG (mmol/L)	10.81±2.30	9.16±2.20	2.844	0.006*
Fating insulin (mU/ L)	11.19±1.84	7.88±0.91	8.859	<0.0001*
Triglycerides (mmol/L)	2.80±0.85	2.23±0.55	3.068	0.003*
Total cholesterol (mmol/L)	5.44±0.84	5.65±0.63	–1.071	>0.05
LDL-C (mmol/L)	2.88±0.68	2.54±0.73	1.901	>0.05
HDL-C (mmol/L)	1.46±0.41	1.81±0.42	–3.287	0.002*
HOMA-IR	2.74±0.51	1.47±0.25	12.388	<0.0001*
FABP4 (ng/ml)	1.47±0.25	0.20±0.07	27.094	<0.0001*
PTEN (ng/ml)	6.46±1.57	4.72±0.82	5.685	<0.0001*

* Indicates that the difference between two groups is of significance. GDM – gestational diabetes mellitus; ΔBMI – BMI before delivery abstracts Pre-pregnancy BMI; FABP4 – adipocyte fatty acid binding proteins; PTEN – phosphatase and tensin homolog deleted on chromosome ten; HOMA-IR – homeostasis model assessment- insulin resistance; FPG – fasting plasma glucose; 2hPG – 2 hour plasma glucose.

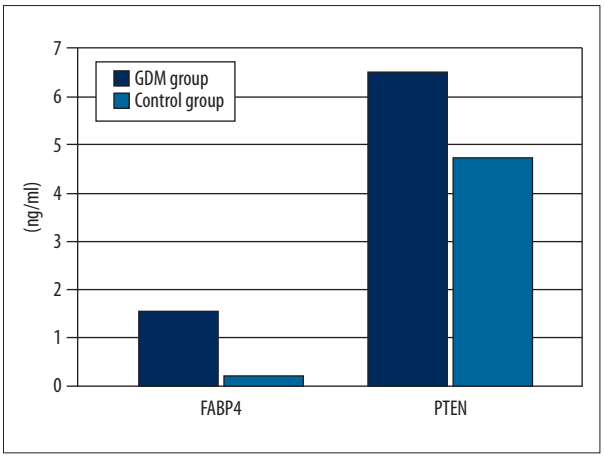

Figure 1. Plasma levels of FABP4 and PTEN in the GDM and control groups. FABP4: Plasma fatty acid-binding protein-4; PTEN: Phosphatase and tensin homolog.

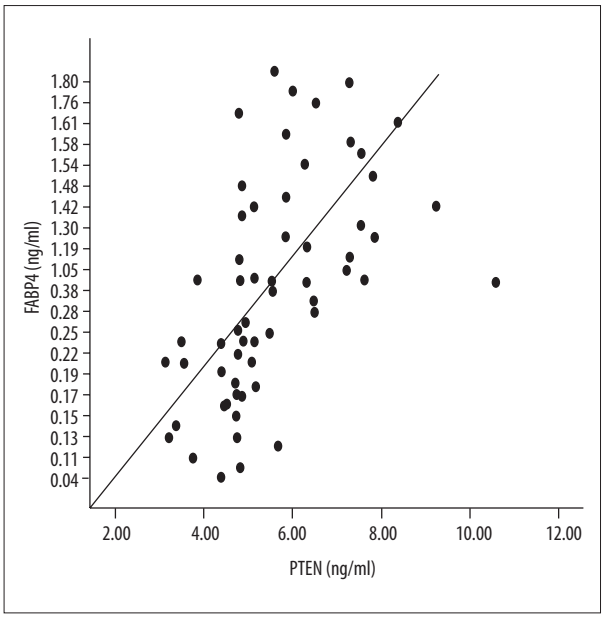

Figure 2. Positive relationship between plasma FABP4 and PTEN. FABP4: Plasma fatty acid-binding protein-4; PTEN: Phosphatase and tensin homolog.

to controls. No significant differences in TC and LDL-C were found between the 2 groups.

The plasma FABP4 in the GDM and control group was 1.47±0.25 *vs.* 0.20±0.07 ng/ml (*P<0.0001*), respectively; the PTEN in the GDM and control group was 6.46±1.57 *vs.* 4.72±0.82 ng/ml, *P<0.0001*, respectively (Figure 1). When all samples (including the GDM and control groups) were statistically analyzed, there was a positive relationship between PTEN and FABP4 (r=0.64,

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Table 2. The influence of factors on HOMA-IR by multiple regression analysis.

Model	Unstandardized Coefficients		Standardized coefficients	t	Sig.
	B	Std. eroor	Beta		
(Constant)	0.484	0.163		2.971	0.004
FABP4	0.807	0.072	0.712	11.200	0.000
TG	0.224	0.057	0.227	3.888	0.000
PTEN	0.070	0.035	0.138	1.976	0.053
BMI			0.119	1.397	0.168
ΔBMI			–0.080	–1.444	0.154
2hPG			–0.045	–0.888	0.378
TC			–0.014	–0.271	0.787
LDL			0.009	0.180	0.858
HDL-C			0.029	0.531	0.598

Dependent Variable: HOMA-IR.

P<0.0001) (Figure 2). In the GDM group, there was positive relation between plasma FABP4 and HOMA-IR (r=0.566, *P*=0.001). A similar relation were also found between PTEN and HOMA-IR (r=0.542, *P*=0.002) in the GDM group.

In the GDM group, the influence of parameters such as BMI, ΔBMI, TG, TC, LDL-C, HDL-C, 2-hPG, and FINS on HOMA-IR was further investigated by multiple linear regression analysis, which showed that FABP4 and TG are 2 significant independent factors influencing HOMA-IR, while the effect of PTEN on HOMA-IR was nearly significance (*P*=0.053, Table 2). The multiple linear regression equation was $Y=0.484+0.807x_1+0.224x_2+0.07x_3$ (x_1: FABP4; x_2: TG; x_3: PTEN; Y: HOMA-IR), R=0.931, R^2=0.867, F=121.882 (*P<0.001*).

Discussion

We found that the GDM group had a higher HOMA-IR than the control group, which is consistent with previous studies [15–17]. Several case-control studies found that increased insulin resistance is associated with abnormalities in body weight, blood glucose, lipids, and lipoprotein. For example, higher pre-pregnant BMI is coupled with increased risk of GDM [18]. Another study showed that the FPG, 2-hPG, FINS, 2-hFINS, TG, LDL-C, and HOMA-IR in GDM patients were significantly higher than that of euglycemic pregnant women and a positive relation was found between pregnant age, pre-pregnant BMI, and HOMA-IR [19].

Our study also found that the GDM patients had a higher concentration of FABP4 compared to the controls. Furthermore, the multiple regression analysis revealed that plasma FABP4 is an independent risk factor for insulin resistance. In a study by Ortega et al., the GDM group (n=98) had a higher FABP4 level than the control group (n=86), 19.9±1.0 *vs.* 17.7±0.8 ng/mL, *P*=0.0493 [20]. These results consistently indicate that a higher level of FABP4 is associated with increased insulin resistance and may play a significant role in the pathogenesis of GDM [20–22].

As an important intracellular fatty acids carrier protein, FABP4, is released from adipocytes and plays an important biologic role in fatty acid uptake, transport, and metabolism [23]. FABP4 may influence insulin sensitivity and energy metabolism by regulation of fatty acid metabolism. Increased FABP4 can promote the accumulation of short-chain fatty acids in cells and decrease PI3K-AKT protein activity, thereby inhibiting glucose oxidation and glycolysis and significantly reducing glucose uptake and utilization in muscle and liver [24–27]. Therefore, the pathway from glucose to triglyceride is disturbed and the increased insulin resistance may lead to GDM [24–27]. However, why adipocytes in GDM patients produce higher levels of FABP4 is still unknown.

The relation between GDM and the plasma levels of PTEN has not been investigated. Our study found for the first time that GDM patients had a significantly higher level of PTEN than normal controls and that this is positively associated with HOMA-IR, indicating that the increased PTEN levels may also contribute to the pathogenesis of increased insulin resistance.

PTEN, as a tumor-suppressive protein, has been widely investigated in recent years, but its role in insulin sensitivity and glucose metabolism is largely unknown. Increased expression of PTEN may result in more severe insulin resistance by blocking the intracellular insulin-signaling pathway [22], catalyzing

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phosphatidylinositol-3,4,5-triphosphate (PIP3) degradation [28], and inhibiting the transportation of glucose into the cells. In PTEN knockout experimental animals, insulin sensitivity is increased in liver, muscle, and adipose tissue [29–32]. Cowden disease, caused by a PTEN gene inactive mutation, also shows improved insulin sensitivity [33]. All these findings strongly suggest that the lost function of PTEN can increase insulin sensitivity.

Our study showed that there is a positive correlation between plasma FABP4 and PTEN, indicating that the interaction of these two factors may cause more severe insulin resistance. A recent study revealed that a complex of FABP4 and PTEN was found in differentiated adipocytes, showing that the complex may enhance the potential activity of PTEN and result in more severe insulin resistance [13], but the underlying mechanisms need further investigation.

Our study had some limitations. As a cross-sectional study, we could not make a cause-and-effect conclusion between higher levels of FABP4, PTEN, and increased insulin resistance. Furthermore, we did not know whether the higher levels of FABP4 and PTEN already existed before the pregnancy or whether they extend after delivery. Prospective longitudinal studies are required to answer these questions.

Conclusions

GDM patients had more severe insulin resistance than the control group, partially contributed to by elevated plasma FABP4 and PTEN. The net increment of body weight during pregnancy and dyslipidemia were independent factors for insulin resistance.

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