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Endogenous galanin as a novel biomarker to predict gestational diabetes mellitus

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ABSTRACT

Although a significantly higher level of plasma galanin was found in patients with gestational diabetes mellitus (GDM) in our previous study, it is unknown whether plasma galanin is biomarker for the prediction of GDM. The present study aims to further evaluate the relationship between endogenous galanin and GDM in pregnant women and to find out the precise mechanism by which galanin plays role in the pathogenesis of GDM. The study registered thirty pregnant women with GDM and thirty pregnant women with normal glucose tolerance (NGT). Demographic and biochemical parameters and fasting venous blood samples of two groups were collected from all cases. Galanin was analyzed by an enzyme-linked immunosorbent assay. Gamma-glutamyl transferase (GGT) was measured by enzymatic methods. The plasma galanin and GGT levels were found higher in GDM compared with NGT ($P<0.001$). In addition, a significant positive correlation was shown between galanin and fasting glucose ($P=0.049$), 1-h glucose ($P=0.033$), body mass index (BMI) ($P<0.001$) and GGT ($P=0.048$) in pregnant women with GDM, whereas there was significant positive correlation between galanin and BMI ($P=0.030$) in NGT group. The plasma galanin and GGT levels are higher in patients with GDM. The plasma galanin levels appear to be related to the changes of blood glucose, BMI and GTT in GDM. The higher level of galanin observed in GDM may represent a adaptation to the rise of glucose, weight, GGT associated with GDM. The higher level of plasma galanin is a novel biomarker for the prediction of GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of varying severity with onset or first recognition during pregnancy [24]. There are numerous factors involved in pathogenesis and mechanisms of GDM, including an increase in food intake and body weight as well as a progressive increase in glucose and insulin resistance [6]. Due to the ongoing worldwide epidemic of GDM [6], there is a pressing need to predict and evaluate the GDM precisely and promptly. In contrast to the wealth of work for type 2 diabetes, as recently reviewed [11], the literature on effective biomarker for prediction of GDM is less mature. Thus, further exploration of novel biomarker responsible for GDM from clinical utility is urgently needed.

Currently, there are a wealth of evidences demonstrate that the neuropeptides are involved in the regulation of energy metabolism and glucose homeostasis. In particular, galanin is one of these important neuropeptides that reduces insulin resistance and improves glucose uptake in animals and humans [11]. Galanin, a 29/30-amino-acid peptide, was first isolated in 1983 from porcine intestine by Tatemoto and collaborators [33]. It is synthesized in the central and peripheral nervous system, and also commonly expressed in peripheral tissues to modulate food intake, energy metabolism, reproduction and pain threshold [12,14,34].

Recent studies provided clues on the relationship between galanin and glucose homeostasis in humans and animals [13,14]. First, elevated plasma galanin levels were found in patients with obesity, type 1 diabetes mellitus, type 2 diabetes and GDM [8,10,12,20]. During a glucose tolerance test, galanin secretion in healthy volunteers and type 2 diabetic patients is positively correlative with the blood glucose level, which is dependent on insulin sensitivity [20,21]. Besides, a significant positive correlation between galanin level and blood glucose concentration or body mass index (BMI) was found in women with GDM compared with controls [10]. In addition, in children with type 1 diabetes mellitus, a positive correlation was found between galanin and hemoglobin A1c (HbA1c) [8], which may be an important predictive factor in GDM pregnancies. Second, galanin can directly

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inhibit glucose-stimulated insulin release in animals and humans [23,28,32]. However, the inhibitory effect of galanin on insulin secretion does not interfere its ability to benefit insulin sensitivity of subjects. Third, the diabetic rats have an obvious reduction in plasma galanin and galanin-immunoreactive cells in the pancreatic islets compared with non-diabetic rats [1]. During glucose tolerance tests, galanin or GalR1 gene-knockout mice experienced impaired glucose disposal caused by a reduction in insulin response and insulin-independent glucose elimination [2,36], while the homozygous galanin transgenic C57BL/6J mice with the obese phenotype showed an increase in the metabolic rates of lipid and carbohydrate [27]. Finally, our and Bu et al. studies indicated that administration of M35, a galanin antagonist, reduced 2-deoxy-[3H]-D-glucose (2-DG) contents in myocytes and adipocytes, and glucose infusion rates in the hyperinsulinemic euglycemic clamp test which was a direct assessment of insulin sensitivity in animals [7,15,16,18,22,35]. Besides, administration of M35 also regulated circulating glucose levels by stimulating glucose uptake through the acceleration of the translocation of glucose transporter 4 to the plasma membrane of various insulin-sensitive cells [7,15,16,18,22,35]. Therefore, these studies provide convincing evidence that galanin is an important hormone that increases insulin sensitivity and promotes glucose transportations in humans and animals.

Based on these recent studies, it appears that galanin might have a role in regulating glucose, insulin, insulin sensitivity and HbA1c metabolism and it also might have a role in the of pathogenesis GDM. Although elevated plasma galanin levels were found in patients with GDM in our previous study, it is unknown whether the higher level of plasma galanin is biomarker for prediction and risk of GDM. The present study aims to further evaluate the relationship between endogenous galanin and GDM in pregnant women and to find out the precise mechanism by which galanin plays role in the pathogenesis of GDM.

2. Material and methods

The present study was conducted in the Clinical Medical College, Yangzhou University, after the approval of the ethics committee dated February 2013. The present study consisted of 30 women at 24–28 weeks of gestation with GDM and 30 women at 24–28 weeks of gestation with normal glucose tolerance (NGT). And all subjects not previously diagnosed with overt diabetes and without a family history of diabetes. Women with preeclampsia and other pregnancy complications (except GDM) were excluded from the study. Written consents of all participants were obtained and the protocol of the study was approved by the local ethics committee (Clinical Medical College, Yangzhou University).

"One-step" method (according to the International Association of Diabetes and Pregnancy Study Groups criteria) has been employed to diagnose whether or not it is GDM, as described previously [17]. In brief, all subjects were given 75 g oral glucose tolerance test (OGTT) at 8:00 A.M. after overnight fasting of at least 8 h. And plasma glucose were measured at fasting, 1 and 2 h [17]. The diagnosis of GDM is made when any of the following plasma glucose values are exceeded [fasting plasma glucose threshold of 92 mg/dL (5.1 mmol/L), 1 h plasma glucose threshold of 180 mg/dL (10.0 mmol/L) and 2 h plasma glucose threshold of 153 mg/dL (8.5 mmol/L)] [17].

Plasma glucose concentrations were measured using oxidase method. Plasma insulin levels were measured by radioimmunoassay (China Institute of Atomic Energy, Beijing, China). HbA1c was evaluated by a high performance liquid chromatography technique (HPLC VariantTM, Bio-Rad, Germany). HOMA-IR index was calculated for each patient using the formula [fasting glucose (mmol/L) × fasting insulin (μIU/mL)/22.5]. For each case body mass index (BMI) was calculated at the time of blood collection as weight in kilograms divided by height in meters squared.

Table 1
Demographic and biochemical characteristics of study and control groups.

	NGT	GDM	*P* value
N	30	30	
Age (years)	25.67 ± 1.83	26.9 ± 2.82	0.05
Gestational Age (weeks)	25.1 ± 1.45	25.43 ± 1.01	0.305
BMI (kg/m^2)	23.69 ± 2.47	25.95 ± 2.46	0.001
HbA1c (%)	4.75 ± 0.46%	5.39 ± 0.72%	<0.001
Fasting glucose (mmol/L)	4.33 ± 0.39	6.18 ± 1.13	<0.001
1-h glucose (mmol/L)	7.19 ± 1.22	12.88 ± 2.84	<0.001
2-h glucose (mmol/L)	6.63 ± 1.17	12.92 ± 3.17	<0.001
Fasting Insulin (μIU/mL)	12.35 ± 2.03	15.82 ± 3.53	<0.001
1-h Insulin (μIU/mL)	18.51 ± 1.82	21.95 ± 5.83	0.004
2-h Insulin (μIU/mL)	14.01 ± 3.86	27.00 ± 5.70	<0.001
HOMA-IR	2.38 ± 0.46	4.38 ± 1.41	<0.001

Results are shown as means ± SD; Statistical significance $P < 0.05$; *N*, number of cases.

3. Blood sample handling and peptide assay

Fasting venous blood samples were collected from all cases in the study in the first visit (upon diagnosis) in order to determine levels of galanin, GGT, glucose, fasting plasma insulin, HbA1c. The blood samples (3 mL) were collected in prechilled EDTA tubes containing aprotinin and were immediately centrifuged for 15 min at 1000 × *g* at 4 °C within 30 min of collection [10]. Plasma was separated into vials and stored at −80 °C until measurement. Galanin was analyzed by an enzyme-linked immunosorbent assay (Uscn Life Science, Inc., Wuhan, China). According to the manufacturer's specification, the range of the assay was 12.35–1000 pg/mL, and the average sensitivity was 4.21 pg/mL. Gamma-glutamyl transferase (GGT) was measured by enzymatic methods using an automated biochemistry analyzer (Roche DDPP, Germany). All measurements were performed in duplicate, and the mean of the two measurements was considered.

4. Statistical analysis

Statistical analysis was performed using the SPSS statistical software for Windows (Version 17.0). Data for each respective study were presented as mean ± SD. The differences between the groups were analyzed with independent *t*-test. Possible correlations between parameters were evaluated by Spearman's correlation coefficient analyses. $P < 0.05$ was regarded as statistically significant.

5. Results

The study registered 30 pregnant women with GDM and 30 pregnant women with NGT. The results showed no significant differences in age (25.67 ± 1.83 vs. 26.90 ± 2.82, $P = 0.05$) and gestational weeks (25.10 ± 1.45 vs. 25.43 ± 1.01, $P = 0.305$) between the pregnant women with GDM and NGT, whereas BMI, fasting glucose, 1-h glucose, 2-h glucose, fasting insulin, 1-h insulin, 2-h insulin, HbA1c and HOMA-IR were significantly higher in the pregnant women with GDM compared with NGT (see Table 1). Furthermore, the statistically significant higher levels of galanin and GGT were found in pregnant women with GDM compared with NGT (25.69 ± 3.38 pg/mL vs. 18.56 ± 2.90 pg/mL, $P < 0.001$; 31.80 ± 7.38 U/L vs. 20.30 ± 3.72 U/L, $P < 0.001$) (see Figs. 1 and 2).

In addition, there were no significant correlations between galanin and any of the anthropometric or metabolic parameters studied in NGT group except of BMI ($r = 0.518$, $P = 0.030$) and 2-h insulin ($r = -0.458$, $P = 0.011$). Interestingly, there was no