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Original Article

Circulating betatrophin in pregnant individuals with gestational diabetes mellitus and normal glucose tolerance

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Abstract: The role of betatrophin in diabetes has been paid attention; however, there were controversial conclusions. This study was aimed to investigate serum levels of betatrophin in pregnant participants with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM), as well as the relationships between its circulating concentrations and some clinical parameters. Between August 2014 and April 2015, a total of 150 women subjects including 50 female healthy controls, 50 NGT participants, and 50 GDM patients were recruited. All the subjects were age-and pre-body mass index (BMI)-matched, along with matched gestational weeks between the NGT and GDM group. Serum betatrophin levels were analyzed using sandwich enzyme linked immunosorbent assay (ELISA). Also, serum levels of triglycerides (TG), cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) were analyzed. Moreover, Pearson correlation analysis was applied to determine the correlations between the parameters and betatrophin levels. Serum betatrophin levels were significantly higher in the NGT and GDM subjects than health controls ($P<0.001$). But no significance was found in the NGT and GDM with respect to all the parameters in our study. In addition, betatrophin levels were positively correlated with pre-BMI, ALT, and AST in the health controls, and positively correlated with pre-BMI in the GDM group, but negatively correlated with AST in the NGT group. These results indicate that serum betatrophin levels were associated with insulin resistance (IR) induced by pregnancy in NGT and GDM.

Keywords: Betatrophin, gestational diabetes mellitus, normal glucose tolerance pregnancy

Introduction

Gestational diabetes mellitus (GDM) has long been recognized and defined as an unclearly overt diabetes with any degree of glucose intolerance during pregnancy [1]. It has been reported that GDM affects 1-14% of the pregnant women according to the population-based studies [2], particularly common in China and India [3], and the frequency is estimated to continue to grow [4]. Moreover, it has been well demonstrated that women who suffer from GDM have a major risk factor for developing type 2 diabetes in their later life (incidence from 2.6%-70%) [5, 6], and the risk factor for developing type 1 diabetes is also raised [7]. In addition, pregnancy-associated risk has also been increased. Therefore, new developments in the pathogenesis and treatment of GDM are of importance.

Insulin resistance (IR) occurs to some degree in all pregnancies [8], and both IR and an impaired insulin secretion are reported to be associated with GDM [9]. Recently, a newly liver- and fat-derived hormone was discovered by Yi and coworkers [10], betatrophin (also named lipasin, angiopoietin-like 8 protein (ANGPTL8), refeeding-induced fat and liver protein (RIFL)) has been paid extensive attention due to the association with IR and proliferation of pancreatic beta cells. However, there have been disputes about betatrophin on the proliferation of pancreatic beta cells, about the associations between betatrophin levels and BMI, between betatrophin levels and lipid profiles or insulin sensitivity [10-14]. Hence, further studies should be performed to determine the exact results. Studies concerned on betatrophin in the pathogenesis of metabolic disorders (e.g. type 1 and 2 diabetes, obesity, and lipid metab-

Table 1. Characteristics of the study subjects

Variable	Healthy controls (N=50)	NGT (N=50)	GDM (N=50)	P^1 value	P^2 value	P^3 value
Age (years)	28.6±1.51	28.62±1.72	28.63±1.72	0.843	0.531	0.414
Gestation (days)	NA	275.78±7.22	278.63±5.52	NA	NA	0.127
Pre-BMI (kg/m^2)	20.61±1.70	21.42±1.61	20.94±1.78	0.199	0.645	0.361
Fasting glucose (mmol/l)	4.81±0.17	4.15±0.43	3.97±0.33	<0.001	<0.001	0.104
Fasting insulin (uU/ml)	4.65±1.20	8.05±2.19	8.02±2.12	<0.001	<0.001	0.966
HOMA-IR	0.99±0.26	1.49±0.44	1.41±0.39	<0.001	0.003	0.561
TG (mmol/l)	0.63±0.21	1.54±0.39	1.46±0.38	<0.001	<0.001	0.493
CHO (mmol/l)	4.41±0.71	4.61±0.62	4.45±0.75	0.428	0.216	0.43
HDL-C (mmol/l)	1.81±0.23	1.87±0.59	1.69±0.38	0.073	0.315	0.574
LDL-C (mmol/l)	2.44±0.61	2.57±0.61	2.23±0.47	0.553	0.92	0.517
ALT (U/L)	21.30±14.40	14.78±4.45	15.25±3.55	0.19	0.222	0.688
AST (U/L)	23.50±9.00	21.03±5.46	24.38±6.86	0.425	0.796	0.097
AKP (U/L)	64.6±11.92	160.43±51.57	142.00±30.29	<0.001	<0.001	0.112
Betatrophin	288.22±115.30	1027.93±490.53	1020.65±390.45	<0.001	<0.001	0.954

NGT, normal glucose tolerance; GDM, gestational diabetes mellitus; N, number; NA, not available; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; CHO, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and AKP, alkaline phosphatase. P^1 value, comparisons between NGT and health controls; P^2 value, comparisons between GDM and health controls; and P^3 value, comparisons between NGT and GDM. Statistical analysis isapplied using independent sample t-test.

olism) have been sprung up [15-17]. However, little information is available regarding the associations between the function of betatrophin in pregnant participants with normal glucose tolerance (NGT) and gestational diabetes mellitus (GDM). Therefore, we aimed to investigate serum betatrophin levels in pregnant participants with NGT and GDM, as well as the relationships between its circulating concentrations and some clinical parameters.

Materials and methods

Study population

A total of 150 participants were recruited in our study from August 2014 to April 2015 including 50 normal glucose tolerance (NGT) pregnant participants, 50 age-, gestational weeks-, and pre-body mass index (BMI)-matched gestational diabetes mellitus (GDM) participants, and 50 healthy age-matched normal controls. We performed an oral glucose tolerance test (OGTT) to all enrolled subjects. GDM was diagnosed by an OGTT at 24-28 weeks of gestation, which was based on the Australasian Diabetes In Pregnancy Society (ADIPS) guidelines(either a fasting plasma glucose level of ≥5.5 mmol/L and/or ≥8.0 mmol/L[2] hours after a 75 g oral glucose load) [18]. The women who were diagnosed with GDM accepted dietary and physical activity suggestions given by the hospital's dietitian, and glucose were well controlled. All the pregnant participants were their first pregnancy. Individuals with type 1 or 2 diabetes, ≥ second pregnancy, and some known major diseases (e.g. active hepatitis/liver cirrhosis) were excluded from our study. Informed written consent was obtained from all patients. The studies concerning human subjects were in agreement with the guidelines of the Ethics Committee of Affiliated Zhongshan Hospital of Dalian University.

Measurement of biochemical components and anthropometric parameters

Peripheral venous blood samples were drawn from all the enrolled participants after 10 h overnight fasting at the time of OGTT. The blood samples were allowed to clot at 20°C and centrifuged at 1000 ×g at 4°C for 15 min. After separation, the blood samples were stored at refrigerator at -80°C until use. Fasting glucose levels were measured using hexokinase method, fasting insulin levels were determined using chemiluminescence; serum triglycerides (TG), cholesterol (CHO), high-density lipoproteincho-

Table 2. The correlation between betatrophin and clinical parameters

Betatrophin	Health controls (N=50)		NGT (N=50)		GDM (N=50)	
	R	P	R	P	R	P
Age (years)	0.703	0.014	0.016	0.924	0.005	0.986
Gestational (weeks)	NA	NA	-0.235	0.161	-0.18	0.504
Pre-BMI (kg/m^2)	0.806	0.03*	-0.171	0.312	0.512	0.042*
Fasting glucose (mmol/l)	-0.389	0.267	0.294	0.077	0.13	0.63
Fasting insulin (uU/ml)	0.023	0.949	-0.075	0.658	0.142	0.599
HOMA-IR	-0.035	0.923	0.027	0.873	0.165	0.542
TG (mmol/l)	0.025	0.946	0.184	0.277	-0.203	0.45
CHO (mmol/l)	-0.124	0.734	-0.263	0.116	0.161	0.551
HDL-C (mmol/l)	-0.18	0.618	0.257	0.124	-0.36	0.17
LDL-C (mmol/l)	0.147	0.684	-0.086	0.614	-0.203	0.452
ALT (U/L)	0.866	0.001**	-0.082	0.63	0.246	0.358
AST (U/L)	0.784	0.007**	-0.036	0.042*	-0.124	0.648
AKP (U/L)	-0.307	0.388	0.026	0.88	-0.171	0.526

NGT, normal glucose tolerance; GDM, gestational diabetes mellitus; N, number; NA, not available; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglycerides; CHO, cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and AKP, alkaline phosphatase *P<0.05; **P<0.01. Pearson correlation analysis is performed to determine possible correlations.

lesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were assessed using enzymatic analysis, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (AKP) were analyzed by enzymatic activity rate method. Quantitative analyses of serum betatrophin levels were performed using sandwich enzyme linked immunosorbent assay (ELISA, Wuhan Eiaab Science, Wuhan, China) according to the manufacturer's instructions. All samples were prepared in duplicate. The samples with a more than 15% coefficient of variation (CV) were excluded.

For the anthropometric measurements, BMI, expressed as kg/m^2, was calculated by dividing weight in kilograms by the square of height in meters. Gestational weeks were assessed beginning from the first day of the last menstrual period or by using ultrasound dating (for persons whose last menstrual period was unclear).

Statistical analysis

The data, expressed as mean ± standard deviation (SD), were analyzed by statistical package for the social sciences (SPSS) (version 17.0; SPSS Inc., Chicago, IL). Student's t test was used to statistical comparisons. One-way analysis of variance (ANOVA) was performed to multiple comparisons. Pearson correlation analysis was applied to determine a possible correlation between each parameter and betatrophin levels. A statistical significance was defined when P<0.05.

Results

Characteristics of the study subjects

The clinical characteristics of the three groups were showed in **Table 1**. There were no significant differences in age and pre-BMI among the three groups due to the matched selection, as well as in gestational weeks between the NGT and GDM group. In addition, no significant differences were found in CHO, HDL-C, LDL-C, ALT, and AST among the three groups. Moreover, there were no statistically significant differences between the NGT and GDM group with respect to fasting glucose, fasting insulin level, HOMA-IR, AKP, and betatrophin concentration. But statistically significant differences were observed between the healthy controls and NGT group, and between healthy controls and GDM group with respect to fasting glucose, fasting insulin, HOMA-IR, TG, AKP and betatrophin (all P<0.01).

Correlation between betatrophin and clinical parameters

The correlations between betatrophin and clinical parameters were summarized in **Table 2**. As shown in the table, we found that there were strong positive correlations between betatrophin level and pre-BMI (R=0.806, P=0.03), ALT (R=0.866, P=0.001), AST (R=0.784, P=0.007) in health controls. Moreover, strong positive correlations were observed between betatrophin level and pre-BMI (R=0.512, P=0.042) in GDM group. But there were negative correlations between betatrophin level and AST (R=-0.036, P=0.042) in NGT group.

Discussion

The present study demonstrated that both serum betatrophin levels in NGT pregnant participants and GDM patients were significantly higher than those in healthy controls. In addition, we found that serum betatrophin levels were positively correlated with pre-BMI, ALT, and AST in the health controls, and positively correlated with pre-BMI in the GDM group, but negatively correlated with AST in the NGT group.

GDM has been considered as the most frequent type of diabetes among in pregnancy. The incidence of this disease has been increasing worldwide, partly due to the increase of obesity [19]. Hence, it is of importance to find out more suitable and effective treatments that focus on the pathogenesis of GDM-damaged insulin secretion and IR. However, the IR induced by pregnancy is still unknown. An animal experiment found that the amount of insulin degraded by placenta is raised, resulting in acceleration of insulin removal [20]. Besides, IR can be facilitated by various changes of hormone and metabolism during the second half of pregnancy [21]. Betatrophin has been well demonstrated that betatrophin derives from liver, white adipose tissue (WAT) and brown adipose tissue (BAT) [22], but the function is still controversial. Concentrations of betatrophin in humans were firstly measured by Espes, and the betatrophin concentrations were found approximately more 50% higher in type 1 diabetes patients than that in controls [15]. Besides, Fu [16] and Hu both found that circulating betatrophin levels were significantly increased in type 2 diabetes subjects. Moreover, a recent study indicated that serum betatrophin levels in patients with type 2 diabetes were statistically higher than those in NGT subjects [23]. Additionally, researchers have found that betatrophin levels were elevated in GDM patients [24, 25]. Similarly, we also found that the serum betatrophin levels were not only increased in GDM patients but also in NGT subjects. However, there were no significant differences in serum betatrophin levels between NGT pregnant participants and GDM patients. The well-controlled glucose in the GDM patients may contribute to the results. Moreover, there were significant differences in fasting glucose, fasting insulin, TG level, AKP level, and HOMA-IR between NGT pregnant participants and healthy controls, and between GDM patients and healthy controls. The results may be associated with metabolic changes during pregnancy. The pregnant subjects in our study were all the third trimester when the adipose tissue depots of maternity reduce, and the ability of glucose disposal mediated by insulin deteriorates by 40-60% compared with pre-pregnancy [26]. Besides, insulin's ability to suppress the whole body lipolysis is also declined during late pregnancy [27], and notably further reduced in GDM patients [28], devoting to the increase of hepatic glucose, and severe IR [29].

In addition to the above results, the relationships between betatrophin circulating concentrations and some clinical parameters were also investigated. The results demonstrated that betatrophin levels were positively correlated with pre-BMI, ALT, and AST in the health controls, and positively correlated with pre-BMI in the GDM group, but negatively correlated with AST in the NGT group. However, our results were partly inconsistent with previous studies. A study conducted by Fenzl suggested that betatrophin concentrations were associated with LDL-C and TC, but not with BMI [14]. Whereas, Espes indicated that BMI, HDL-C and TG were all not associated with betatrophin [17]. The paradoxical results may be associated with the following factors: (1), all the enrolled subjects in our study were all lean (pre-BMI <25 kg/m^2), and obesity or overweight subjects were not recruited. However, the BMI in the previous study was different, such as the BMI >30 kg/m^2 or 18-28 kg/m^2 [23]. (2), all GDM patients in our study had well-controlled glucose level, but some newly diagnosed type 2 diabetes patients received no drugs [30] or received different drugs [14]; (3), the selection of betatrophin kits may also contribute the conflicts. The kits made by Eiaab and Phoenix Pharmaceuticals used different antibodies [31]. (4), the participants in our study were all female, but in previous studies male and female were both included. Lastly, the race and dietary habit may also be the reasons for the dispute.

In conclusion, our results demonstrate that serum betatrophin levels are significantly increased in pregnant participants with NGT and GDM, indicating that serum betatrophin levels are associated with IR induced by pregnancy in NGT and GDM.

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Disclosure of conflict of interest

None.

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