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The changes of neutrophil gelatinase-associated lipocalin in plasma and its expression in adipose tissue in pregnant women with gestational diabetes

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ABSTRACT

Aims: To investigate plasma levels and the expression of neutrophil gelatinase-associated lipocalin (NGAL) in subcutaneous adipose tissue (SAT) in patients with gestational diabetes mellitus (GDM).

Methods: The study recruited 260 Chinese women divided into three groups: 96 were healthy pregnant women with pre-pregnancy body mass index (pre-pregnancy BMI) below 25 kg/m^2 (GROUP 1), 84 were women with GDM with pre-pregnancy BMI below 25 kg/m^2 (GROUP 2) and 80 were women with GDM with pre-pregnancy BMI over 25 kg/m^2 (GROUP 3). Laboratory and anthropometric measurements were recorded and NGAL plasma levels were determined by ELISA for subjects in all groups. Real-time RT-PCR and Western blotting were used to assess the relative mRNA and protein expression of NGAL and tumor necrosis factor-α (TNF-α) in SAT (30 cases in each group).

Results: Our results demonstrated statistically significant elevation in plasma NGAL concentrations in GROUP 2 and GROUP 3 compared with GROUP 1 ($p < 0.001$ for both group comparisons). Moreover, SAT NGAL mRNA ($p < 0.001$ and $p < 0.001$, respectively) and protein ($p < 0.001$ and $p < 0.001$, respectively) expression levels were higher in GROUP 3 than in both GROUP 1 and GROUP 2. Correlations were noted between the plasma NGAL concentration and various parameters of insulin resistance.

Conclusions: Plasma NGAL may play a role in the development of insulin resistance in GDM, and the high levels of NGAL expression in SAT in overweight women with GDM suggests that NGAL in SAT is associated with obesity in women with GDM.

1. Introduction

Gestational diabetes mellitus (GDM) is characterized by hyperglycemia, hyperinsulinemia and insulin resistance and is associated with the future development of type 2 diabetes mellitus. The pathogenesis of GDM remains unknown but abnormal adipocyte function may play a role in the development of GDM.

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It is recognized that adipose tissue is as an endocrine organ [1] which plays an important role in energy balance [2] as well as metabolic homeostasis [3–5] and is associated with obesity-related chronic low-grade inflammation, insulin resistance and metabolic syndrome. More specifically, adipokines may contribute to insulin resistance and metabolic diseases [6]. Of all the adipokines, neutrophil gelatinase-associated lipocalin (NGAL) is a potential mediator that links chronic low-grade inflammation to obesity.

NGAL (or lipocalin-2), a 25-kDa glycoprotein belonging to the superfamily of lipocalins, was first discovered in human neutrophils [7] and is expressed in many tissues such as liver, kidneys and adipose tissue. NGAL has been demonstrated to be an inflammatory marker closely interrelated to insulin resistance and hyperglycemia [8].

To elucidate the relation between insulin resistance and NGAL during pregnancy, we examined plasma levels of NGAL and its local concentrations in subcutaneous adipose tissue (SAT) among women with GDM and healthy pregnant controls. Moreover, we investigated the correlation between NGAL and tumor necrosis factor-α (TNF-α), a predictor of insulin resistance in human pregnancy [9].

2. Material and methods

2.1. Subjects and tissue collection

Subjects were recruited from pregnant women who had routine prenatal examinations in the outpatient clinic and were later hospitalized for cesarean section delivery. Indications for cesarean delivery included previous cesarean delivery, breech presentation, fetal macrosomia and social factors. All participants had undergone screening for GDM with a 75 g oral glucose challenge test at 24–28 weeks' gestation, according to the 2011 criteria of the American Diabetes Association [10] in the period from January 2011 to May 2013. Those with multiple pregnancies, type 2 diabetes mellitus, hyperthyroidism or hypothyroidism, Cushing's disease, and chronic inflammatory disorders such as Crohn's disease, collagen tissue disorders, renal, liver, cardiovascular disease, and infectious diseases were excluded. The study included 260 women of whom 80 women had GDM with their pre-pregnancy body mass index (pre-pregnancy BMI) over 25 kg/m^2 (GROUP 3), 84 had GDM with pre-pregnancy BMI below 25 kg/m^2 (GROUP 2) and 96 healthy pregnant women with pre-pregnancy BMI less than 25 kg/m^2 with similar age and gestation weeks as control group (GROUP 1). Plasma NGAL levels were measured in all subjects. NGAL mRNA and protein levels in SAT were estimated in 30 random subjects from each group. Adipose tissue samples were obtained from subjects who underwent lower segment cesarean section. All subjects were informed of the study and gave written informed consent. The study was approved by the institutional review board of Shenzhen Bao'an Maternal and Child Health Hospital.

A standardized questionnaire was used to collect basic information. Anthropometric measurements such as height, weight, and pre-pregnancy weight were recorded and pre-pregnancy BMI was calculated by dividing weight (in kilograms) by height (in meters) squared. After an overnight fast (8–10 h), blood samples were obtained from all the subjects. Subcutaneous adipose tissue (0.5 cm × 0.5 cm × 0.5 cm) was sampled on the operating table when subjects were having cesarean delivery. Plasma were separated and stored at −80 °C. The adipose tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C.

2.2. Measurement of metabolic parameters

Fasting plasma glucose (FPG), fasting plasma insulin (FPI), high-sensitivity C-reactive protein (hs-CRP), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured by an automatic analyzer (Olympus AU640, Japan). Insulin resistance was calculated using the homeostasis model assessment ratio, HOMA-IR = [FPG (mmol/L) × FPI (μU/ml)/22.5].

2.3. ELISA quantification of NGAL and TNF-α

Plasma TNF-α concentration was quantified using Quantikine ELISA System (R&D Systems, Minneapolis, MN). Plasma NGAL levels were assessed by the quantitative sandwich enzyme immunoassay technique (Quantikine Human Lipocalin-2/NGAL Immunoassay; R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation were 3.7% and 6.5%, respectively.

2.4. RNA isolation and real-time quantitative PCR analysis

Total RNA was extracted from subcutaneous adipose tissue samples following the manufacturer's instructions using Trizol (Invitrogen, USA) and was digested with DNase I (Promega, USA) for removing any trace of genomic DNA. First-strand cDNA was synthesized using M-MLV reverse transcriptase (Promega, USA) and random hexamers (Promega, USA) as primers. Since the amount of template cDNA and the number of cycles were determined experimentally, quantitative comparison could be made during the exponential phase of the amplification process. The cDNA was amplified at the following conditions: 50 °C for 2 min, 95 °C for 2 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 32 s. Quantitation of NGAL and TNF-α gene were performed via utilizing SYBR Green qPCR Super Mix (Invitrogen, USA) and an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Foster City, USA). Our study used GAPDH as an internal standard. The following primers were used for our study: NGAL (120 bp), 5-CAAGGAGCTGACTTCGGAACT-3 and 5-TGCACTCAGCCGTCGATACA-3; TNF-α (131 bp): 5-TTCTGCCTGCTGCACTTTG-3 and 5-TGGGCTACAGGCTTGTCACT-3; GAPDH (299 bp): 5-GGGAAACTGTGGCGTGAT-3 and 5-GAGTGGGTGTCGCTGTTGA-3. (All the primers were synthesized by Shenggong, Shanghai, China.)

2.5. Western blotting

Tissue samples were homogenized on ice in a Triton X-100 cell lysis buffer (Biyuntian, China). Proteins derived from tissue lysates, acquired by using the Bradford method, were