Original Article
Correlation of resistin expression in maternal serum and subcutaneous adipose tissue with insulin resistance in gestational diabetes mellitus

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Abstract: Objective: To investigate the expression of resistin (RETN) in maternal serum and subcutaneous adipose tissue, and their relationship with insulin resistance (IR) in gestational diabetes mellitus. Methods: From February 2011 to November 2012, 43 cases of definite GDM (gestational diabetes mellitus) patients with cesarean section at 37-40 gestational weeks were selected as GDM group, and they were divided into satisfactory control group and unsatisfactory control group. 24 healthy pregnant women of 37-40 weeks at the same period were defined as control group. The serum levels of resistin, fasting plasma glucose (FPG), fasting insulin (FINS) of all pregnant women were measured by ELISA, glucose oxidase method, electrochemiluminescence immunoassays respectively. The Home model insulin resistance index (HOMA-IR) was calculated. Realtime-PCR and Western Blot were applied to investigate the expression of RETN mRNA, protein in subcutaneous adipose tissue. The relationships of the serum levels of resistin and insulin resistance in two groups were compared. Results: The full-term body weight and BMI were significantly higher in unsatisfactory control group than that of satisfactory control group and the control group (P<0.05), and there were significantly differences among the 3 groups for the FINS and HOMA-IR (P<0.05). The mRNA expressions of RETN in adipose tissue of pregnant women in GDM group were both higher than those of the control group. In a certain range, as resistin mRNA and protein expression increased, IR level also showed a significant upward trend. Conclusion: Resistin is probably an important cytokines in GDM.

Keywords: Resistin, gestational diabetes mellitus, insulin resistance

Introduction

Gestational diabetes mellitus (GDM) is a common complication during pregnancy, which is defined as abnormal glucose metabolism that only occurs during pregnancy. The incidence of GDM is worldwide reported of 1%-14% and is rising year by year [1]. 25%-85% of GDM patients will appear all sorts of complications, such as preeclampsia, polyhydramnios, macrosomia, fetal malformation and stillbirth, and the newborns are prone to occur respiratory distress syndrome (RDS), hypoglycemia, hypocalcemia and polycythemia, etc. Therefore, GDM is of great harm to mothers and infants [2], which attracts more attention from Chinese and foreign obstetrics and gynecology. The causes of GDM are relatively complex. Due to the lack of appropriate animal models and the limitations of human experimentation, the specific pathogenesis of GDM remains unclear. Currently, islet β cell function defect and insulin resistance (IR) are considered to be the key link during onset of GDM [3] and about 17-63% of GDM patients develop into type 2 diabetes in postpartum 5-13 years [4], so it is speculated that the pathogenesis of GDM is similar to that of type 2 diabetes. IR involves many factors, where resistin (RETN) secreted by adipose tissue may play an important role [5].

At present the relationship between resistin and GDM is controversial. Some literatures reported that resistin was significantly increased in GDM patients and was considered to play an important role in the pathogenesis of GDM [6]. And some reports [7, 8] showed that there were no significant differences between normal preg-
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...nancy and GDM patients in blood resistin or in resistin of cord blood. It was speculated that resistin was not obviously related to GDM and did not affect the fetal energy metabolism. The different results may be related to the object of gestational age and the effect of blood sugar control. In this study, resistin expressions in serum and abdominal subcutaneous adipose tissues of pregnant women with gestational diabetes mellitus (GDM) after well-controlled blood glucose at full-term pregnancy were detected to discuss the relationship between resistin and insulin resistance (IR) and the roles of resistin in the development of GDM.

Materials and methods

43 cases of definite GDM patients with cesarean section at 37-40 gestational weeks were selected as GDM group, and they were divided into satisfactory control group with 26 cases and unsatisfactory control group with 17 cases. All of the patients were from maternity clinic of Shanghai Pudong Hospital between February 2011 and November 2012. Diagnostic criteria referred to diagnostic criteria for gestational diabetes industry in 2011 (the ministry of health). And 24 cases of healthy pregnant women with cesarean section at 37-40 gestational weeks were randomly selected as the control group. The three groups of pregnant women were collected for general information including age, gestational week, height and weight (full-term pregnancy and pre-pregnancy), and body mass index was calculated (BMI = body weight (KG)/body height (M²)). All of the three groups of pregnant women were single birth, without other complications. The patients did not take any drug that can interfere with glucose and lipid metabolism, such as indo-methacin, phentolamine, furosemide, thiazide diuretics, phenytoin sodium and cortisone, etc.

GDM therapy and criteria of satisfactory blood glucose control

After GDM diagnosis (the the 43 cases of definite GDM patients), patients received gestational nutrition education, started diet control and appropriate sports. Calculated the total calories the pregnant woman needed per day according to body height, body weight and pregnancy week. Sugar accounted for 50-60% of the total calories, protein 15-20% and fat 20-30%. Implemented frequent smaller meals, daily caloric intake of breakfast, lunch, and dinner were 15%, 30%, 30%, intakes between meals and bedtime snacks were 5%, 10%, 10%. And then if they both satisfy the ideal criteria of which weekly review of fasting and post-prandial 2-hour blood glucose were less than 5.3 mmol/l and less than 6.7 mmol/l, they would be selected into satisfactory control group. Otherwise, they would be unsatisfactory control group. In case of a still higher blood glucose than normal after diet control for 1-2 weeks, we treated the pregnant woman with insulin and the insulin dosage was 0.5-0.7 U/kg.d. Starting from a small dose, the gradually increased the dosage of the insulin according to the blood glucose level until blood glucose was ideally controlled.

Sampling and determination

Sampling: (1) Blood sampling: All the research objects had fasting phlebotomized in the 24 hours before the cesarean section. And 4 mL fasting blood was taken from cubital vein and centrifuged for serum. Then fasting blood glucose and insulin were detected immediately. The rest serum was preserved at -20°C. When all the specimens were collected, serum resistin of same batch was determined. (2) Adipose tissue sampling: About 1 cm³ of abdominal subcutaneous adipose tissue was taken for all the patients undergone cesarean section surgery, the specimens were rinse with sterilized saline, and dried with the sterilized gauze. Then they were placed into the 1.5 mL of sterile centrifuge tubes, which was preserved in refrigerator at -80°C for RNA and protein extraction.

ELISA, glucose oxidase (GOD) and electrochemical luminescence were respectively used for determination of serum resistin, FPG and FINS. ELISA kits of same batch were purchased from Shanghai Guantai Biological Technology Co. LTD. Batch variation was less than 5%, and testing procedures were conducted in strict accordance with the specifications.

Real-time-PCR was used to detect mRNA expressions of RETN in adipose tissue: Trizol (invitrogen) was used to extract total RNA in accordance with the specification, reverse transcription kit (takara) was used to synthesize cDNA, and SYBR-Premix Ex-Taq reagent (takara) and LightCycle 480 (Roche) for quantitative analysis of mRNA. Primers are designed as fol-
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Primers were synthesized from Shanghai Bioreys. 2-ΔCT values were used to represent the relative amounts of mRNA.

Western Blot was used to detect protein quantity: Total protein of adipose tissue was extracted with Tissue Total Protein Lysis Buffer (Beyotime) according to the specifications. And BCA kit (Beyotime) was used determine protein concentration, which was boiled for degeneration after adding sample buffer. 45 μg protein was given electrophoresis separation on with 12% SDS-PAGE and then transferred to PVDF membrane (Millipore) by semi-dry transfer unit (Biorad). After 2 h of blocking with NET block liquid, the specimens were added with mouse-anti-human primary antibody of 1:1000 (Santa Cruz) and stayed overnight. Membranes were washed with TBST for 4 times, with 5 min each time. HRP-labeled goat-anti-rat secondary antibody of 1:5000 (Santa Cruz) was added. And then the membranes were incubated for 1 h and washed. After 5 min of incubation with ECL luminous fluid, the images were created on imager (Tanon). Image was used to analyze grey value of electrophoresis band.

Evaluation indexes of insulin resistance: Homoeostasis model assessment of insulin resistance (HOMA-IR) index was calculated, by the method of (FINS×FPG)/22.5. And it was used as evaluation index of IR.

Statistical methods

SPSS13.0 was used for statistical analysis. Measurement data was represented as mean ± standard deviation (SD) (x±s). One-way ANOVA was used to compare the groups and then LSD was used for multiple comparisons, while Spearman correlation analysis was used for correlation between two variables. We define the statistical inspection level α=0.05.

Results

Comparisons among 3 groups of pregnancy women in general data

There were no statistical differences between the three groups in pregestational weight and body mass index (BMI) (P>0.05). But full-term body weight and BMI were significantly higher in unsatisfactory control group than that of satisfactory control group and the control group (P<0.05), as shown in Table 1.

Comparisons among 3 groups of pregnancy women in serum resistin, FINS, FPG and HOMA-IR

There was no significantly difference between the satisfactory control group and the control group for resistin and FPG (P>0.05), but the resistin and FPG of the two groups were significantly lower than the unsatisfactory control group (P<0.05). What's more, Of the FINS and HOMA-IR, there were significantly differences among the 3 groups by multiple comparisons (P<0.05), and it was the unsatisfactory control group, satisfactory control group and the control group aranging from high to low. As shown in Table 2.

mRNA and protein expressions of RETN in adipose tissues of 3 groups of pregnant women

In order to more clearly reveal the roles of RETN in the pathogenesis of GDM, we adopted Realtime-PCR which is relatively precise in

<table>
<thead>
<tr>
<th>Items</th>
<th>GDM with Satisfactory control (26 cases)</th>
<th>GDM with Unsatisfactory control (17 cases)</th>
<th>Control group (24 cases)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>26.82±3.44</td>
<td>28.41±4.3</td>
<td>27.95±4.3</td>
<td>1.576</td>
<td>0.099</td>
</tr>
<tr>
<td>Pregnant week (weeks)</td>
<td>38.7±1.46</td>
<td>37.1±1.37</td>
<td>38.9±1.65</td>
<td>0.233</td>
<td>0.801</td>
</tr>
<tr>
<td>Body height (M)</td>
<td>1.60±0.04</td>
<td>1.62±0.04</td>
<td>1.58±0.04</td>
<td>0.635</td>
<td>0.543</td>
</tr>
<tr>
<td>Progestation body weight (KG)</td>
<td>53.95±7.77</td>
<td>54.54±7.83</td>
<td>54.08±8.24</td>
<td>0.358</td>
<td>0.674</td>
</tr>
<tr>
<td>Progestation BMI (KG/M²)</td>
<td>21.35±7.66</td>
<td>21.18±7.85</td>
<td>21.05±2.28</td>
<td>0.557</td>
<td>0.544</td>
</tr>
<tr>
<td>Full-term body weight (KG)</td>
<td>67.04±7.97*</td>
<td>70.09±9.33*</td>
<td>66.65±7.98*</td>
<td>3.875</td>
<td>0.021</td>
</tr>
<tr>
<td>Full-term BMI (KG/M²)</td>
<td>26.53±2.89*</td>
<td>27.22±3.51*</td>
<td>25.94±2.78*</td>
<td>4.001</td>
<td>0.018</td>
</tr>
</tbody>
</table>

*Compared with the control group, P<0.05, *Compared with the Unsatisfactory control group, P<0.05.
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<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Resistin (μg/ml)</th>
<th>FPG (mmol/l)</th>
<th>FINS (μu/ml)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDM with Satisfactory control</td>
<td>26</td>
<td>16.561±4.78*</td>
<td>4.44±0.66*</td>
<td>22.97±3.87*</td>
<td>4.86±0.87*</td>
</tr>
<tr>
<td>GDM with Unsatisfactory control</td>
<td>17</td>
<td>23.33±5.01</td>
<td>5.09±0.58</td>
<td>27.09±3.35</td>
<td>6.13±0.80</td>
</tr>
<tr>
<td>Control group</td>
<td>24</td>
<td>14.57±4.89</td>
<td>4.43±0.67</td>
<td>20.33±4.22</td>
<td>3.68±0.73</td>
</tr>
</tbody>
</table>

Table 2. The comparison of three groups of pregnant women of resistin, FINS, FPG, HOMA-IR

At the same time, we detected these specimens at protein level to intuitively exhibit corresponding protein expressions in adipose tissue. And the results showed that protein expressions of GDM with unsatisfactory control group were also higher than those of the control group, with statistically significant difference, as shown in Figures 2 and 3.

**The correlation among insulin resistance index, serum and adipose tissue**

There was no significantly correlations between serum resistin and HOMA-IR (r=0.265, P>0.05); And there were positive correlations between resistin mRNA and protein with HOMA-IR expressions in subcutaneous adipose tissue (r=0.575, 0.851, P<0.05). In a certain range, as resistin mRNA and protein expression increased, IR level also showed a significant upward trend.

**Discussion**

Correlation between pregnant women with GDM and insulin resistance

More and more researches showed that from the metabolic and endocrine aspects, GDM is...
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a kind of decompensated state due to the combined effects of physiological IR and chronic IR in late pregnancy that cannot be overcome by the compensatory secreted insulin of islet beta cells [9]. This kind of chronic IR may already exist before pregnancy. And after pregnancy, under the effects of placental hormone secretion, IR is further strengthened while the amount of insulin secretion is limited, so the increase of insulin is not enough to offset the pregnancy-induced IR and blood glucose is increased from the middle-late stage of pregnancy thus resulting in GDM [5]. This study showed that even the blood glucose was well controlled in GDM group, insulin and HOMA-IR were also significantly increased compared to the control group. It was promoted that peripheral insulin target organ of GDM patients had severer IR than the normal pregnancy women. And it was also suggested that IR was an important cause for GDM. The GDM pregnant women of 37~40 pregnant week still showed a higher level of HOMA-IR and FINS than those in healthy control group after the satisfactory control of blood glucose by controlling diet, exercise and insulin therapy, indicating the damage of islet β-cells which provided insulin and modulated the glucose metabolism in pregnant women was not permanent. These cells maintained the normal glucose metabolism by increasing the secretion of insulin against insulin resistance. Insulin resistance existed not only in midtrimester but in the full-term pregnancy when blood glucose was satisfactorily and persistently controlled and further affected the pregnancy outcome of pregnant women with GDM.

Correlation between serum resistin level of GDM and BMI

Resistin was first found in 2001 by Steppan et al. [10] in their study on mechanism of new-type anti-diabetic drugs such as thiazolidinedione, which was named as resistin for its association with IR. Most scholars believe that resistin can lead to IR, which is associated with obesity and type II diabetes, etc. [11]. Changes of serum resistin level of GDM patients were still controversial. Causes for different results may be related to the gestational age, blood glucose control status, BMI, the severity of the disease and the presence of complications, etc. This study showed that in 37-40 weeks of full-term gestation, BMI level and serum resistin were both decreased in the satisfactory control group compared to unsatisfactory control group, but there were no significant difference compared to the healthy control group. Azuma et al. [12] followed up 64 cases of obesity people and found that 35 cases had lower serum resistin after 1.5-year of exercise and diet, and the result was positively correlated with BMI. High-fat diet can significantly increase resistin level in mice, which gain weight and present as GDM; resistin is also increased in ob/ob fat mice with genetic obesity characteristics and in db/db diabetic mice with genetic diabetic characteristics [9]. It was speculated that after diet, exercise or insulin treatment, GDM pregnant women could control blood glucose to satisfactory range. Their body weight grow was slowed down, synthesis of adipose cells and secretion of resistin were decreased, thus serum resistin level was decreased.

Expression of the adipose tissue and clinical significance

Resistin is mainly expressed in subcutaneous, mammary gland and epididymis white adipose tissue, etc, while is few expressed in brown adipose tissue. Human resistin is also expressed in adipose tissue, placenta, and bone marrow tissue and blood mononuclear cells [13]. Reports about resistin expression changes in adipose tissue of GDM pregnant women were extremely rare. This study showed that resistin mRNA and protein expression levels in GDM pregnant women with unsatisfactory blood glucose control were significantly higher than those of normal pregnant women. And resistin mRNA and protein expressions were obviously correlated with HOMA-IR. This was suggested that resistin of adipose tissue participated into the IR of GDM patients, which was similar to the related literature at home and abroad [14, 15]. But this study did not find obvious correlation between serum resistin changes of GDM and HOMA-IR in pregnant women. This may be because adipose tissue is not the only source of resistin, and placenta can also secrete resistin during pregnancy. Resistin is expressed in the human placenta [16]. And it may induce IR through the following mechanisms, thus leading to GDM: (1) through induction of SCOS-3
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expression, reduction of Akt activity and damage on glucose to stimulate insulin secretion [17, 18]; (2) through down-regulating expression of IR on beta cells to influence I function and activity of beta cells [19].

Resistin is probably an important cytokines in GDM. Further research on the physiological role of resistin and its action mechanism, may be of great significance in prevention and treatment of GDM and in improvement of maternal and infant prognosis, and may provide a new sensitive index in clinical evaluation on IR degree of GDM patients.

Acknowledgements

This research was approved by the institutional review board (CWO) of Pudong Hospital Affiliated to Fudan University, and all the patients had provided the written informed consent.

Disclosure of conflict of interest

None.

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References

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