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Expression of PI3-K, PKB and GSK-3β in the skeletal muscle tissue of gestational diabetes mellitus

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ABSTRACT

Objective: To analyze the expression of phosphatidylinositol 3 kinase (PI3-K), protein kinase B (PKB) and glycogen synthase kinase 3 beta (GSK-3 β) in skeletal muscle tissue of gestational diabetes mellitus (GDM). **Methods:** A total of 90 cases of pregnant women were divided into observation group and control group according to the occurrence of GDM with 45 cases in either, and the expression of PI3-K, PKB, GSK-3 β mRNA expression in skeletal muscle tissue was compared between two groups. **Results:** The total PI3-K p85 protein was significantly higher in the observation group compared with the control group, the activity of PI3-K was lower than that of the latter; The total PKB, GSK-3 β protein in skeletal tissue had no significant difference between two groups, while the serine phosphorylation levels of PKB and GSK-3β were significantly lower in observation group compared with the control group. **Conclusions:** The downregulation of PI3-K, PKB and GSK-3β in skeletal tissue of GDM caused by phosphorylation dysfunction of signaling molecules is the reason for insulin resistance and transporter function decline which lead to GDM.

1. Introduction

Gestational diabetes mellitus is (GDM) a special type of diabetes mellitus, referring to the impaired glucose intolerance and insulin resistance (IR) which has severe adverse effects on pregnant woman and fetus[1-3]. Mortality of GDM tents to increase rapidly along with the improvement of living standards. Previous reports have reported that the occurrence of IR is related to post insulin receptor signaling transduction disorder[4,5]. We analyzed the critical signaling molecules of receptors in skeletal tissue including phosphatidylinositol 3 kinase (PI3-K), protein kinase B (PKB) and glycogen synthase kinase 3 beta (GSK-3β) to explore the mechanism of GDM[6-8].

2. Materials and methods

2.1. Clinical materials

A total of 90 pregnant women were selected. Inclusion criteria was as follows: Sigleton pregnancy; accepted prenatal examinations and caesarean. Exclusion criteria was as follows: Pregestational diabetes mellitus; taken drugs which can affect the matabolism of glood glucose and blood lipid before pregnancy[9,10]; had complication with other diseases such as hypertension, heart disease, renal disease and liver disease. Patients were included in this research after signing informed consent and were divided into observation group and control group. There were 45 patients

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in observation group diagnosed by glucose screening test and oral glucose tolerance test[11,12], age was 22-34 years old with average (28.4±6.1), body mass index (BMI) was (21.4±1.1) kg/m^2, gestational week were 37-40 weeks with average (39.0 ±0.8) weeks, times of pregnancy was 1-2 times with average (1.3±0.2) times; There were 45 patients in control group, age was 21-34 years old with average (29.0±7.3), BMI was (20.9± 1.4) kg/m^2, gestational week were 37-40 weeks with average (38.8±0.7) weeks, times of pregnancy was 1-2 times with average (1.4±0.2) times. Age of pregnant women, BMI, and gestational week had no statistical significantdifference ($P>$ 0.05). The research had statistical comparability.

2.2. Research methods

2.2.1. Sample collection

A total of 200 mg fectus abdominis was kept during cesarean delivery and was divided into 2 parts treated by 4% triformol and liquid nitrogen for detection respectively. The whole procedure abided by sterilized operation.

2.2.2. Detection method

SP method was applied for immunohistological staining to detect the expression of PI3-K, PKB and GSK-3β in skeletal tissue of pregnancy women, claybank color reaction under microscope represented positive, primary antibodies were not added in control group, average optical density values of PI3-K, PKB and GSK-3β in different fields of vision were calculated by HPIAS-1000 image analysis system to be compared[13,14].

Western Blot was used to extract protein samples and detected the protein density, and then electrophoresis, exposure and fixation were applied after protein density was modified to standard density. Serine phosphorylation of PKB and GSK-3β was detected and density value of electrophoretic bands were calculated by gel imaging and analysis system.

ELISA was applied to detect PI3-K activity. 4% triformol fixed muscle tissue was used in SP method and Western Blot , liquid nitrogen fixed muscle tissue was used in ELISA.

2.2.3. Image processing

Photoshop CS5 was chosen to process the images and each sample was processed for 2 times to obtain the mean value; Image grey value was analyzed by Gene Tools Analysis Software[16,17].

2.3. Statistical analysis

All date in our study were analyzed by SPSS13.0. Enumeration data was analyzed by *Chi*-square test and measurement data was analyzed by t test. The test level was set as α =0.05. The difference was considered as statistically significant when $P<0.05$.

3. Results

3.1. PI3-K expression

Distribution of PI3-K p85 protein in skeletal tissue of two groups was observed in immunohistological staing, as shown in Figure 1A and B; Total amount of PI3-K p85 protein was significantly higher in observation group (1.52±0.03) compared with control group (1.01±0.02) (t=4.230, P<0.05), and the activity of PI3-K was lower in observation group (0.84±0.11 *vs*. 2.36±0.17, t=5.579, P<0.05).

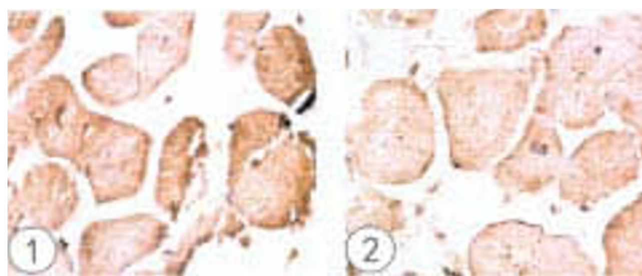

Figure 1. Expression and distribution of PI3-K.
1: observation group (magnification times: 200; 2: control group (magnification times: 200.

3.2. PKB expression

The total PKB protein in two groups showed no statistical difference (1.22±0.13 *vs*. 1.17±0.10) (t=0.385, $P>0.05$), Serine phosphorylation of PKB was significantly lower in observation group compared with control group (0.31±0.08 *vs*. 0.62±0.13) (t=0. 4.907, P<0.05) (Figure 2).

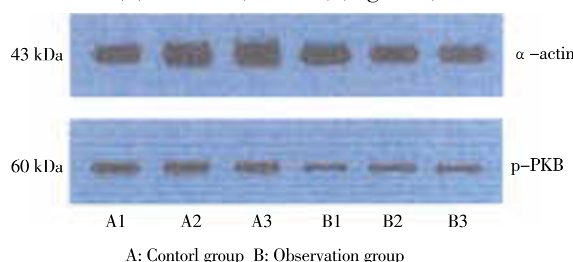

Figure 2. Serine phosphorylation level of PKB in skeletal tissue between two groups.

3.3. GSK-3β expression

The total GSK-3β protein in skeletal tissue had no significant difference between two groups (0.98±0.05 *vs*. 1.02 ±0.07) (t=0.039, $P>0.05$), Serine phosphorylation level of GSK-3β was lower in observation group compared with control group (0.30±0.03 *vs*. 0.62±0.07) (t=2.977, $P<0.05$).

4. Discussion

Insulin sensitivity decreased in pregnant women to reduce the consuming of parent glucose and satisfy the need of fetus, however, over decrease of insulin sensitivity in some pregnant women can cause GDM mainly with IR change which can harm safety of maternal and child[18-21]. Foreign scholars consider that the mechanisms include produce of anti-insulin antibody, mutation of insulin receptor and dysfunction of post insulin receptor signaling pathway [22,23], any of the above problems can cause incidence of GDM.

PI3-Kpathway and RAS mitogen activated protein kinase are the main pathways of post insulin receptor signaling transduction. Recent years most of researches have considered that PI3-K pathway which is related to metabolic regulation is the critical step that affects the regulation of glucose and lipid level in body[24,25]. In our research we found that the total PI3-K p85 protein in observation group was significantly higher compared with the control group, and activity of PI3-K was lower, which was related to the regulation defect of PI3-K p85 gene expression in GDM patients. Long term stimulation of high density insulin can cause the over expression of p85 subunit, cause upregulation of PI3-K p85 protein, has negative feedback on insulin sensitivity, and affect the activity of downstream molecules to inhibit the continuous conduction of signal[26]. Meanwhile, we found the total PKB and GSK-3β proteins had no statistical difference, however serine phosphorylation levels of PKB and GSK-3β in observation group were significantly lower in observation group compared with control group, the main mechanism: PKB is phosphorylation activated by upstream PI3-K. After activation of PI3-K is decreased, the phosphorylation state of PKB serine phosphorylation site is changed so that transporter function is changed to inhibit the transporting function of glocuse[27,28]. However the total PKB protein had no obvious change, indicating that there was no relation between the degradation of PKB and GDM. Meanwhile, insulin signal continuously activates PI3-K and downstream glycogen synthase by phosphorylating serine site of GSK-3β. Decrease of PI3-L and PKB activity can decrease the activation of GSK-3β on downstream signaling molecules, which affects the synthesis of glycogen and cause increase of blood glucose level[29]. Besides, El *et al*[30] pointed that GSK-3β could phosphorylate serine and threonine residues of IRS-1 and reduce the activation effect of PKB to further affect the transduction of insulin signaling. In conclusion, expression downregulation of PI3-K, PKB and GSK-3β in skeletal tissue of GDM caused by phosphorylation dysfunction of signaling molecules is the critical reason that causes function defect of IR and transporter to further causes GDM.

Conflict of interest statement

We declare that we have no conflict of interest.

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