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Association of low maternal levels of salusins with gestational diabetes mellitus and with small-for-gestational-age fetuses

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ABSTRACT

Objectives: To evaluate maternal and cord serum concentrations of salusin-α and salusin-β in women with gestational diabetes mellitus (GDM) and with small-for-gestational age (SGA) fetuses.
Study design: Pregnant women with GDM ($n = 25$), women with SGA ($n = 20$) and maternal age-matched normal healthy pregnant subjects ($n = 25$) participated in the study. Maternal serum and cord blood salusin-α and salusin-β levels at the time of birth were measured using ELISA, and their relation with metabolic parameters was also assessed.
Results: Mean concentrations of maternal and fetal serum salusin-α in the GDM and SGA groups were significantly lower than those of the controls ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively). Mean concentrations of maternal and cord blood salusin-β also decreased in both the GDM and the SGA groups in comparison to the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$ and $P < 0.001$, respectively). The concentrations of maternal serum salusin-α and salusin-β were strongly positively correlated with the concentrations of cord blood salusin-α and salusin-β ($R = 0.92$, $P < 0.001$ and $R = 0.94$, $P < 0.001$, respectively).
Conclusions: The low levels of maternal serum salusin-α and salusin-β may have negative impact on metabolic disorders and vascular dysfunction.

1. Introduction

Many recent studies have concentrated on the vascular endothelium as a possible target organ in gestational diabetes mellitus (GDM) and disease related to placental insufficiency such as small-for-gestational-age (SGA) [1–4]. A low birth weight appears to be an indicator of fetal adaptations to a suboptimal intrauterine environment. A possible mechanism for fetal adaptive responses, which contribute to SGA, is a negative impact on endothelial function [5].

Endothelial cells have an essential effect on the regulation of vascular tone through the release of vasoactive substances [6]. In pathological pregnancies, such as GDM, SGA or preeclampsia, the synthesis of vasoactive substances is altered, leading to changes in uteroplacental circulation, which could induce slowing of fetal growth and development [7,8].

Salusin-α and salusin-β are recently recognized endogenous bioactive peptides, derived from 28- and 20-amino-acid precursors respectively [9]. Salusins are secreted in various tissues such as blood vessels, kidneys, monocytes and macrophages, as well as being detected in human body fluids [9]. They mainly play a role in the cardiovascular system. An experimental study has indicated that infusion of either salusin-α or salusin-β results in low blood pressure and a marked decrease in heart rate and cardiac output [10]. It has also been reported that salusins may play a key role in promoting mild proliferation in vascular smooth muscle and fibroblast cells, and inhibit cardiomyocyte apoptosis [11]. Salusin-β, however, has more potent effects than salusin-α.

The development of vascular endothelial dysfunction may be relevant in women with gestational diabetes who suffer from increased insulin resistance and SGA [12,13]. We therefore evaluated salusin-α and salusin-β levels in such women. The objective of this study was to evaluate maternal and cord serum concentration of salusin-α and salusin-β in women with GDM, SGA and normal healthy pregnancies.

2. Materials and methods

Twenty-five pregnant women who were diagnosed with GDM and 20 pregnant women with fetal SGA diagnosed in the outpatient clinic of the Obstetrics and Gynecology Department

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in Inonu University, Turgut Ozal Medical Center, were recruited as the study groups. From an unselected population of pregnant women undergoing their routine pregnancy follow-up, 25 age-matched pregnant women with normal glucose tolerance test were selected as the control group. The study protocol was approved by the Institutional Ethical Committee for Research on Human Subjects. Informed written consent form was obtained from all the women.

2.1. Participant selection criteria

The inclusion criteria for women with a normal healthy pregnancy were (1) no pre-existing diabetes mellitus, (2) absence of clinical evidence of any major disease such as maternal cardiac disease, connective tissue disorders and renal and liver failure, (3) absence of medical treatment that may alter glucose tolerance, and (4) fetal estimated weight between 10th and 90th customized centiles confirmed at birth.

The inclusion criteria for the pregnant women with GDM were: (1) newly diagnosed GDM cases, (2) no previous use of oral hypoglycemic agents, (3) no history of substance abuse or psychiatric illness, and (4) maternal age between 18 and 40 years. SGA was defined as a fetal estimated weight below 10th centile according to fetal sex, gestational age, maternal parity and reference standards [14] confirmed at birth. Pregnancies were dated according to the first-trimester crown-rump length measurements [15]. In the SGA group, women with gestational hypertensive disease (maternal systolic blood pressure $\geq$ 140/90 mm Hg; significant proteinuria $>$ 300 mg/l/24 h) were excluded.

The exclusion criteria from the study were the presence of (1) pre-existing diabetes mellitus (type-1 and -2), (2) macrovascular and/or microvascular complications, (3) multi-fetal gestation, (4) fetuses with chromosomal, genetic or structural defects and (5) chronic medical diseases such as urolithiasis, cirrhosis, congestive heart failure, hypertensive disorders or other known major diseases.

2.2. Oral glucose tolerance test and diagnosis of GDM

Participants underwent a 1-h 50 g oral glucose challenge test between 24 and 28 gestational weeks as recommended by ACOG [16]. After ingestion of a drink containing 50 g of glucose, venous blood glucose level measured at 1 h. A 100 g oral glucose tolerance test (OGTT) was performed to diagnose gestational diabetes when the blood glucose after the 50 g glucose challenge test was raised (the threshold value is often set as 140 mg/dl). After ingestion of a drink containing 100 g of glucose, venous glucose levels were measured before and at 1, 2 and 3 h. The threshold values were defined as 105, 190, and 165, 145 mg/dl for fasting, 1, 2 and 3-h after 100 g OGTT, respectively. Pregnant women with two or more high serum glucose values were diagnosed as having GDM. Patients with a value of 200 mg/dl or higher after the 50 g glucose challenge test (GCT) were considered to have GDM and did not undergo the 100 g GTT. Normal glucose tolerance was diagnosed when the 50 g GCT value was at or under 140 mg/dl.

Maternal age, body mass index (BMI) at delivery, blood pressure, birth weights, Apgar score and gestational ages at birth were evaluated in the study. Maternal BMI (kg/m^2) was calculated as the ratio of the weight (kg) to the square of the height (m). Maternal blood pressure was measured in the right arm after the participants remained at rest for 10 min, with the subjects being in a sitting position and relaxed. Newborns who were delivered by cesarean section or spontaneous vaginal delivery were weighed, and first and fifth minute Apgar scores were also recorded subsequent to birth.

2.3. Biochemical analysis

Fasting venous blood was obtained from an arm of each woman in the study groups and healthy pregnant woman, after giving birth but before delivery of the placenta. Cord blood samples were obtained from the umbilical cord immediately after delivery from all newborns in the GDM, SGA and control groups. The blood sample was delivered to the laboratory within 20 min, centrifuged (2000 $\times$ g/min for 10 min at 4 °C) and the serum was stored at $-$80 °C until assayed. Serum salusin-α concentration was analyzed using an enzyme-linked immunosorbent assay (ELISA) kit with a minimum detectable concentration less than 4.75 pg/ml, from Uscn Life Science Inc. (Cat No: E9189Hu, Wuhan, P.R. China). The intra- and inter-assay coefficients of variance (CV) for salusin-α ranged from 4.1% to 7.2% and 4.6% to 9.7%, respectively. Serum salusin-β concentration was measured using an ELISA kit with a minimum detectable level less than 8.2 pg/ml, from Uscn Life Science Inc. (Cat No: E92026Hu, Wuhan, P.R. China). The intra- and inter-assay CV ranged from 5.6% to 6.9% and 7.8% to 10.9%, respectively. All samples were read using Bio-Tek Instruments ELx800 Microplate Reader (Vermont, USA). The biochemist was blind to the identity of samples during processing. The results are presented as ng/ml.

Serum insulin levels were measured using a competitive chemiluminescent enzyme immunoassay method with the same trademark kits (Immulite 2000 Analyzer, Diagnostic Products Corporation; DPC, Los Angeles, CA, USA). The respective inter- and intra-assay CV was 5.7% and 4.3% for insulin. Fasting glucose concentration was assessed by enzymatic colorimetric assay methods using an Abbott Architect C16000 auto analyzer (Abbott Diagnostic Lab., USA) and commercially available kits. The inter- and intra-assay CV were 3.4% and 3.0% for fasting glucose. For assessment of insulin resistance, the homeostasis model assessment insulin resistance index (HOMA-IR) was used [17], given as: HOMA-IR = fasting insulin (mU/ml) $\times$ fasting glucose (mg/dl)/405.

2.4. Statistical analysis

Comparison between the outcome groups was performed using chi square test for categorical variables. The normality of distributions was assessed using the Kolmogorov–Smirnov test. Variables (salusin-α and salusin-β in maternal and fetal serum) with a skewed distribution were log-transformed. Since there was a statistically significant difference in the BMI among the SGA, GDM and control groups, comparisons were performed using ANCOVA (BMI and gestational age were as a covariates) for the continuous variables. Correlation analysis was used to determine the significance of association (Pearson's coefficient) between maternal serum and cord blood salusin-α and salusin-β levels with maternal and cord blood insulin, glucose levels, HOMA-IR, maternal age, BMI, gestational age and birth weight in the outcome groups. The results are presented as mean and standard deviation (SD). For all comparisons, a probability of $<$0.05 was considered to be significant. The data were analyzed using the Statistical Package for Social Sciences software 19.0 for Windows package software (SPSS Inc., Chicago, IL).

3. Results

The maternal characteristics of each of outcome groups are compared in Table 1. In comparison with the controls, the mean BMI was significantly higher in the GDM group than the control and SGA groups (P = 0.02 and P = 0.01, respectively). The rate of cesarean section (C/S) was 60.0% (n = 15) in the GDM group, 65.0% (n = 13) in the SGA group and 56.0% (n = 14) in the control group (P = 0.73 and P = 0.49, respectively).