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ORIGINAL ARTICLE

Cord blood nesfatin-1 and apelin-36 levels in gestational diabetes mellitus

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Abstract To assess maternal serum and cord blood apelin-36 and nesfatin-1 concentrations in pregnant women with and without gestational diabetes mellitus (GDM). Thirty pregnant women with GDM and 30 gestational age matched healthy pregnant subjects participated to the study. Maternal serum and cord blood nesfatin-1 and apelin-36 levels were measured with ELISA, at the time of birth. The relationships between maternal serum and cord blood nesfatin-1 and apelin-36 levels, anthropometric and metabolic parameters were also assessed. Maternal serum apelin-36 levels were found higher (13.5 ± 8.3 vs. 9.6 ± 5.9 ng/ml, $P = 0.001$) and nesfatin-1 levels were found lower (5.5 ± 8.1 vs. 8.1 ± 23.9 ng/ml, $P = 0.001$) in patients with GDM compared with control pregnant women. However, the cord blood apelin-36 levels (8.8 ± 4.3 and 8.2 ± 1.9 ng/ml, $P = 0.618$) and nesfatin-1 levels (5.4 ± 4.0 and 6.2 ± 10.3 ng/ml, $P = 0.688$) were similar in the GDM and control groups, respectively. Maternal serum apelin-36 and nesfatin-1 levels correlated positively with their respective cord blood levels. Maternal serum and cord blood apelin-36 levels correlated negatively with the gestational age and birth weight. Similarly maternal serum and cord blood nesfatin-1 levels correlated negatively with the gestational age, but there was no correlation with the birth weight. We did not find a correlation between maternal serum apelin-36 and nesfatin-1 levels, maternal age, BMI, fasting glucose, fasting insulin, and HOMA-IR. Also cord blood apelin-36 and nesfatin-1 levels did not correlate with the maternal age, BMI, HOMA-IR, cord blood glucose, and cord blood insulin levels. Our results indicate that apelin-36 concentrations increase and nesfatin-1 concentrations decrease in maternal serum of women with GDM.

Keywords Gestational diabetes mellitus · Apelin-36 · Nesfatin-1 · Insulin

Introduction

Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy. The prevalence may range from 1 to 14% of all pregnancies, depending on the population studied and the diagnostic tests employed [1–3].

Apelin as an endogenous ligand of APJ receptor, has been described as a new bioactive adipokine, produced and secreted by human mature adipocytes [4]. Apelin peptides are derived from a 77 amino acid precursor, which is processed to several active molecular forms such as apelin-36 or apelin-13 in different tissues and in the bloodstream [5–8]. The physiologically active form is thought to be apelin-36. Apelin serum levels are related to the nutritional status and parallel insulin plasma levels in mice and

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humans [9, 10]. It has been shown that apelin plasma levels are increased in type-2 diabetic patients [9, 11].

Nesfatin-1 is a recently discovered hormone that is derived from the previously described protein nucleobindin-2 (NUCB2) [12]. Previous studies have shown that nesfatin-1/NUCB2 immunoreactive cells are present in a number of discrete neuronal populations, including the hypothalamic arcuate nucleus and paraventricular nucleus, in the brain stem [13]. The novel satiety factor nesfatin-1 has been shown to decrease food intake and body weight [14]. It has been shown that fasting nesfatin-1 levels were significantly lower in type 2 diabetic patients [15] but its effects on GDM are unknown.

Apelin and nesfatin were shown to be expressed in the central nervous system. These markers are involved in the regulation of nutritional status and food intake [13, 16]. In the current study, we aimed to evaluate the role of apelin-36 and nesfatin-1 in the pathogenesis of GDM and also to compare their fetal levels in pregnancies with and without glucose intolerance. We also assessed the association between serum apelin-36 and nesfatin-1 levels and insulin as well as several metabolic parameters in these subjects.

Materials and methods

Thirty pregnant women who got the diagnosis of GDM in the out-patient obstetric polyclinic of the Obstetrics and Gynecology Department were enrolled as the study group. From an unselected population of pregnant women undergoing their routine pregnancy follow-up, 30 gestational age-matched pregnant women with normal glucose tolerance were recruited to the study as the control group. The study protocol was approved by the institutional Ethical Committee for Research on Human Subjects. Informed written consent was obtained from all the women. The inclusion criteria for the normo-glycemic pregnant women were (1) the absence of family history for the type-2 DM, (2) absence of clinical evidence of any major disease, (3) absence of medication use that may alter glucose tolerance. The inclusion criteria for the pregnant women with GDM were: (1) newly diagnosed cases, (2) no previous use of oral hypoglycemic agents. The exclusion criteria from the study were: the presence of (1) type-1 or type-2 diabetes mellitus, (2) macrovascular and/or microvascular complications, (3) urinary tract infections, (4) urolithiasis, (5) liver cirrhosis, (6) congestive heart failure, or (7) other known major diseases. Fifty gram glucose loading test (GLT) was performed for all of the 60 patients who applied between 24th and 28th gestational weeks as recommended by ACOG [17]. A value of 140 mg/dl was considered as the cut-off point and all patients with a value of >140 mg/dl after 50 g GLT underwent a 3 h 100 g glucose tolerance test (GTT) to diagnose GDM. Cut-off values for serum glucose were defined as 105, 190, and 165, 145 mg/dl for fasting, 1-, 2-, and 3-h tests after the 100 g GTT, respectively. GDM was diagnosed if two or more of the serum glucose values were met or exceeded. The patients with a value of 200 mg/dl or higher after 50 g GLT were considered to have GDM and did not undergo 100 g GTT. Normal glucose tolerance was diagnosed when the 50 g GLT value was at or under 140 mg/dl. The 100 g GTT was done early in the morning after an 8–14 h overnight fasting and after an at least 3 days of unrestricted diet (>150 g carbohydrate per day) and unlimited physical activity. The subjects were remained seated and did not smoke throughout the test.

Maternal ages, body mass index (BMI) of the mother at delivery, blood pressure, gestational ages at birth, birth weights, Apgar score were evaluated in the study. All the anthropometric measurements were performed when the patients were in a standing position with joined feets, relaxed abdomen, and arms at their sides. 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 by trained nurses after the subjects had rested for 10 min on the right arm, with the subjects being in a sitting position and relaxed. Cesarean section or spontaneously vaginally delivered newborns were immediately weighed and 1st and 5th min Apgar scores were recorded after delivery.

Biochemical studies

From each GDM women and control pregnant women, fasting venous blood was collected from arm after C/S or vaginal delivery of the baby but before placenta delivery. Cord blood samples were obtained from the umbilical vein immediately after delivery from all GDM and controls. The blood was delivered to the laboratory within 20 min, centrifuged (2,000 g/min for 10 min at 4°C) and the sera aliquoted and stored at −80°C until assayed. In all GDM and control subjects, fasting serum apelin–36 levels were measured by using enzyme immunometric assay (EIA) (Bio-Tek Instruments ELx800 Microplate Reader, Vermont, USA. Kit catalog # EK–057–15, Phoenix Pharmaceuticals, Inc., Burlingame, CA; USA). The minimum detectable concentration was 0.08 ng/ml and the intra- and inter-assay coefficient of variance (CV) ranged from 3.6 to 8.1% and from 5.3 to 8.9%, respectively. Nesfatin-1 levels were measured by using Enzyme-linked immunosorbent assay (ELISA) (Bio-Tek Instruments ELx800 Microplate Reader, Vermont, USA. Kit catalog # E90242Hu, Uscn Life Science Inc., Wuhan, P.R.China). The minimum detectable concentration was 0.192 ng/ml and the

Table 1 Characteristics of the groups

	GDM ($n = 30$)	Control ($n = 30$)	P
Age (year)	30.9 ± 4.2	31.0 ± 3.2	0.945
BMI (kg/m^2)	25.9 ± 3.3	25.7 ± 2.8	0.887
Fasting glucose (mg/dl)	90.7 ± 17.0	93.1 ± 6.9	0.477
Fasting insulin (μIU/ml)	24.5 ± 15.8	12.2 ± 6.6	<0.001*
1-h glucose[a] (mg/dl)	165.1 ± 28.0	104.3 ± 22.1	<0.001*
HOMA-IR	8.5 ± 12.2	3.7 ± 1.5	0.001*
Cord blood glucose (mg/dl)	85.8 ± 16.7	84.7 ± 15.5	0.792
Cord blood insulin (μIU/ml)	24.5 ± 15.8	8.5 ± 0.8	0.114
Gestational age (week)	37.2 ± 2.4	36.7 ± 2.6	0.469
Birth weigt (g)	3002.8 ± 641.7	2653.2 ± 638.8	0.039*
Apgar 1 min	8.2 ± 0.6	8.2 ± 0.9	0.863
Apgar 5 min	8.4 ± 0.9	8.4 ± 2.3	0.941
Cessarean section rate, n(%)	7/30 (23.3%)	3/30 (10%)	0.299
Maternal serum apelin-36 (ng/ml)	13.5 ± 8.3	9.6 ± 5.9	0.001*
Maternal serum nesfatin-1 (ng/ml)	5.5 ± 8.1	8.1 ± 23.9	0.001*
Cord blood apelin-36 (ng/ml)	8.8 ± 4.3	8.2 ± 1.9	0.618
Cord blood nesfatin-1 (ng/ml)	5.4 ± 4.0	6.2 ± 10.3	0.688

Data is given in mean ± standard deviation

* Statistically significant

[a] Glucose level 1 h after 50 g glucose load

intra- and inter-assay CV for nesfatin-1 were 2.9 and 4.0%, respectively.

Serum insulin levels were measured by competitive chemiluminescent enzyme immunoassay method using the same trade mark 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 measured by enzymatic colorimetric assay methods using an Abbott Architect C16000 autoanalyzer (Abbott Diagnostic Lab., USA) and commercially available kits. The inter- and intra-assay CV were 3.4 and 3.0% for fasting glucose. IR was calculated using the homeostasis model assessment insulin resistance index (HOMA-IR) [18], given as: HOMA-IR = fasting insulin (mU/ml) × fasting glucose (mg/dl)/405.

Statistical methods

All statistical analyses were performed by using the SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The normality of distribution of the continuous variables in groups was tested by the Shapiro–Wilk test. The normally distributing variables were analysed with the Student's t-test and the variables that did not show a normal distribution were compared with Mann–Whitney U-test. Cesarean section rates were compared with the Fisher's exact test. Bivariate correlations (computing Pearson's coefficient with their significance levels) between maternal serum and cord blood apelin-36 and nesfatin-1 levels and maternal and cord blood insulin, glucose levels, HOMA-IR, maternal age, BMI, gestational age and birth weight in the GDM and control patients were calculated. The data were presented in mean ± standard deviation (SD) or ratio and percent. For all comparisons, the statistical significance was defined by a P value of <0.05.

Results

The clinical and biochemical characteristics of our subjects are shown in Table 1. The maternal age and BMI of subjects were similar in the groups. GDM women had significantly higher HOMA-IR, fasting insulin, 1-h blood glucose after 50 g glucose overload than control subjects. The mean gestational age was similar in the groups; however, the mean birth weight was significantly higher in the GDM group. Cesarean section rates were similar in the groups. Cord blood glucose and insulin were also similar in the groups. The difference between groups with regard to the 1st and 5 min apgar scores was statistically insignificant. The maternal serum apelin-36 levels were found significantly higher and nesfatin-1 levels were found significantly lower in patients with GDM compared to controls. However, the cord blood apelin-36 and nesfatin-1 levels were similar in GDM and control subjects (Table 1). Maternal serum apelin-36 and nesfatin-1 levels correlated strongly and positively with their respective cord blood levels. Apelin-36 concentration in maternal serum and cord blood were negatively correlated with the gestational age and birth weight (Tables 2, 3). Similarly, maternal serum and cord blood nesfatin-1 levels negatively correlated with the gestational age, but we did not find a correlation of it with the birth weight. Maternal serum apelin-36 and nesfatin-1 levels did not correlate with the maternal age, BMI, fasting glucose,

Table 2 Correlations of maternal serum apelin-36 and nesfatin-1 levels

	Apelin-36		Nesfatin-1	
	r	*P*	*r*	*P*
Age (year)	−0.108	0.410	0.82	0.521
BMI (kg/m^2)	0.139	0.289	−0.046	0.727
Fasting glucose (mg/dl)	−0.060	0.648	−0.004	0.977
Fasting insulin (μIU/ml)	0.134	0.308	0.158	0.228
HOMA-IR	0.054	0.684	0.055	0.674
Gestational age (week)	−0.270	0.037*	−0.324	0.012*
Birth weight (g)	−0.256	0.048*	−0.187	0.153
Cord blood apelin-36 (ng/ml)	0.806	<0.001*	−0.040	0.762
Cord blood nesfatin-1 (ng/ml)	−0.063	0.635	0.958	<0.001*

* Statistically significant

Table 3 Correlations of cord blood apelin-36 and nesfatin-1 levels

	Apelin-36		Nesfatin-1	
	r	*P*	*r*	*P*
Age (year)	−0.149	0.256	0.120	0.362
BMI (kg/m^2)	0.141	0.281	−0.028	0.831
Cord blood glucose (mg/dl)	0.131	0.318	−0.100	0.447
Cord blood insulin (μIU/ml)	0.042	0.751	−0.081	0.537
HOMA-IR	−0.127	0.332	−0.010	0.939
Gestational age (week)	−0.353	0.006*	−0.377	0.003*
Birth weight (g)	−0.315	0.014*	−0.244	0.060

* Statistically significant

fasting insulin, and HOMA-IR. Also cord blood apelin-36 levels and nesfatin-1 levels did not correlate with the maternal age, BMI, HOMA-IR, cord blood glucose, and insulin levels (Tables 2, 3).

Discussion

In the present study we found the maternal serum apelin-36 levels higher and nesfatin-1 levels lower in the GDM group and they correlated positively with their respective cord blood levels. However, the cord blood levels of apelin-36 and nesfatin-1 were similar in the groups. Maternal and fetal apelin-36 concentrations correlated negatively with the gestational age and birth weight. Similarly, maternal and fetal nesfatin-1 concentrations correlated negatively with the gestational age, but it did not correlate with the birth weight.

There is only one study about the apelin level in GDM and it found the circulating apelin level and APJ mRNA expression similar in the pregnant women with GDM and normal glucose tolerance at 24–32 weeks and at term [19]. However, we found it higher in GDM.

The up-regulation of apelin in the GDM women is not clear; it might be the result of either the increased secretion or decreased apelin metabolism. Previous studies have linked the elevated apelin levels to hyperinsulinemia in nonpregnant women [9, 10, 20]. Nevertheless, in normal pregnancy apelin levels increased in early gestation to regulate normal placentation and its levels decreased as the pregnancy progressed, despite increasing insulin resistance [21]. Therefore, insulin resistance does not seem to regulate the apelin levels in pregnancy. Supportingly we and others did not find a correlation of the apelin level with the insulin levels and the insulin resistance index in pregnancies with and without normal glucose tolerance [22]. Another possibility for the elevated apelin-36 levels was the presence of excess of fat tissue due to fat accumulation in pregnancy [10, 11]. Although the BMI of the groups were similar in the current study, we did not measure the amount of subcutaneous fat tissue. Another source of apelin during gestation is the placenta. Cobellis et al. [23] previously showed apelin and APJ receptor expression in the placental tissue. In the current study, elevated apelin level may be due to increased placental production. We found the mean birth weight significantly higher in the GDM group, despite the strict glucose control. We did not weight the placentas after birth; however it may be possible that the placental mass may be bigger in the GDM group which may increase the apelin level in GDM.

The difference in the current and previous results may be due to the difference in the level of blood glucose regulation [19]. Logically the effect of high glucose levels will be most apparent at the term of gestation. However, in the study of Telejko et al., although the number of cases was high enough in the 24–32 weeks, it was not powered enough to detect the difference in the biochemical markers at term.

We have observed that the cord blood apelin-36 levels were significantly correlated with maternal serum apelin-36. It may be possible to speculate that maternal serum apelin-36 levels contribute the majority of cord blood apelin-36 in pregnancy. Another possible explanation is that mother's serum glucose, after its passage via the fetoplacental barrier, induced the release of insulin from fetal pancreas which in turn stimulated the apelin-36 production and secretion from the adipose tissue. However, the fetal glucose, insulin, and apelin-36 levels were similar in the groups and also we did not find a correlation of them. Fetal apelin levels may also originate from the placenta.

We found a negative correlation between the maternal and fetal apelin levels and gestational age and birth weight. This reflects a decreasing level of the apelin-36 level

during gestation. However, we only measured the apelin-36 level once during labor and we did not measure the apelin levels consecutively during the gestation. In rat, it was shown that adipose tissue apelin mRNA expression only increased early in pregnancy [24]. Also in rats, apelin level was found lower in late pregnancy than in mid-pregnancy, due to the accelerated placental metabolism [21].

NUCB2/nesfatin-1 was localized in islet β-cells of the mouse and rat pancreas [25, 26]. In Goto-Kakizaki rats, a type 2 diabetic model characterized with impaired insulin secretion, pancreatic islet nesfatin-1 content was lower [26]. Also NUCB2 mRNA expression in the pancreatic islets was found markedly increased in diet-induced obese mice [27]. Our data together with these reports suggest a possibility that a reduction of nesfatin-1 level in maternal serum of pregnant patients and its insulinotropic action leads to dysregulation of insulin release in GDM patients. In addition, it was recently reported that intravenous injection of nesfatin-1 suppresses hyperglycemia in ob/ob mice by enhancing insulin action [28]. Therefore, nesfatin-1 could act as anti-diabetic factor by enhancing insulin action in pregnant women with GDM.

In the current study we used the two step 50 g glucose loading and 100 g GTTs to diagnose GDM. Although it is a widely accepted approach, there may be some grouping bias which may affect the results of our study. HAPO study has proposed fasting glucose level >91 mg/dl enough for the diagnosis of GDM, to decrease the risk of giving birth to a large for gestational age infant [29]. Given the mean fasting blood glucose was 93.1 mg/dl in the control group; several control cases could be diabetic according to the HAPO criteria.

We strictly controlled the maternal blood glucose levels within the normal range in the GDM group. However, still the mean birth weight was significantly higher in GDM group, possibly due to the lack of consensus on the normal range of blood glucose levels in GDM. Despite the strict control, high glucose levels are expected to cause several physiologic and metabolic effects on fetus, in GDM. However, we did not find a difference in fetal levels of apelin-36 and nesfatin-1 in pregnancies with and without GDM. Therefore, the metabolic effects of GDM on the fetus do not seem to be through these markers.

Our study had some limitations including a small sample size and single measurements of nesfatin-1 and apelin-36 during pregnancy. Further, this is a pilot study powered only to compare nesfatin-1 and apelin-36 levels between these two groups. We were not adequately powered to examine neonatal outcomes. In spite of these limitations we were able to detect higher levels of apelin-36 and lower levels of nesfatin-1 in women with GDM as compared to controls. If these findings can be supported by further studies, then nesfatin-1 and apelin-36 levels may provide a novel approach for identifying women with GDM.

Conflict of interest The authors declare that they have no conflict of interest. The experiments used comply with the current laws of our country in which the study was conducted.

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