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C1q and tumor necrosis factor-related protein 3 is present in human cord blood and is associated with fetal growth

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ABSTRACT

Background: To determine the presence of C1q and tumor necrosis factor-related protein 3 (CTRP3) in cord blood and its relationship with fetal growth among Chinese newborns.
Methods: This pilot study recruited 126 infants (small for gestational age [SGA], n = 34; appropriate for gestational age [AGA], n = 60; large for gestational age [LGA], n = 32); cord blood CTRP3 levels were measured, and fetal growth parameters were collected.
Results: Median (25–75th percentile) CTRP3 levels in the SGA, AGA, and LGA groups were 297.2 (236.4–360.2), 297.5 (261.0–369.9), and 368.6 (298.5–507.1) ng/ml, respectively (P = 0.01). LGA infants had higher CTRP3 levels than AGA infants (multiple linear regression analysis; P = 0.01). The CTRP3 levels were positively correlated with birth weight (r = 0.25, P < 0.01), Ponderal index (r = 0.28, P < 0.01), and placental weight (r = 0.20, P = 0.03) in the total study population. In the subgroup analysis, CTRP3 levels were negatively correlated with birth length z scores (r = −0.39, P = 0.03) and were positively correlated with the Ponderal index (r = 0.43, P = 0.02) only in the SGA group; no other significant correlations were observed. The CTRP3 levels were similar between the sexes (P = NS).
Conclusions: CTRP3 is present in cord blood and might be involved in fetal growth.

1. Introduction

Fetal growth is an important indicator of prenatal and postnatal health. Small for gestational age (SGA) and large for gestational age (LGA) have been associated with a higher risk of adverse outcomes, including infant mortality and morbidity, growth failure, and adult-onset non-communicable diseases and metabolic conditions [1–3]. Although the mechanisms controlling intrauterine growth remain unclear, numerous studies have shown that adipocytokines play important roles in the regulation of fetal growth [4–6] and related adverse outcomes [7,8].

C1q and tumor necrosis factor-related protein 3 (CTRP3; also known as collagenous repeat-containing sequence of 26-kDa protein [CORS26], cartducin, or cartonectin [9]) is a novel adipokine and has structural homologies to adiponectin. Similar to adiponectin, CTRP3 has multiple effects [10]. It acts as an anti-inflammatory adipokine in vitro [11] and inhibits several proinflammatory pathways, including lauric acid-, lipopolysaccharide (LPS)-, and Toll-like receptor (TLR)-mediated inflammation in macrophages and adipocytes [10,12]. Furthermore, CTRP3 is involved in the regulation of metabolism [13]; it can lower glucose levels, inhibit the glucose output in the liver, and improve insulin sensitivity [10,14,15]. Recent studies demonstrated that CTRP3 levels are lower in women with type 2 diabetes, obesity, or hypertension and is negatively correlated with the parameters of insulin resistance [16–18]. Because both inflammation and energy metabolism are influential factors for fetal growth [19], we hypothesized that CTRP3 might be involved in fetal growth. To the best of our knowledge, the relationship between CTRP3 and fetal growth has not been reported. Moreover, there is a lack of research examining the levels of CTRP3 in infants.

2. Materials and methods

2.1. Study population

Newborns born between January 1 and February 29, 2012 at the Guangzhou Women and Children's Medical Center were recruited. The mothers had to be aged ≥18 years and have a singleton live birth. We excluded newborns with congenital anomalies and chromosomal abnormalities or whose mother had pre-pregnancy diabetes or hypertension. The institutional review board of the Guangzhou Women and

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Children's Medical Center approved this study, and all participants provided informed consent.

2.2. Data collection

We collected the following maternal and newborn clinical characteristics from electronic medical records: maternal age, delivery mode, parity, and placental weight, length, width, and thickness as well as the newborn's birth weight, length, and sex. We calculated the birth weight and length z scores for gestational age according to a local reference [20] and a Chinese national reference [21], respectively. Gestational age was confirmed by routine ultrasonography in the first trimester or early second trimester and is reported as the number of completed gestational weeks.

We also calculated the Ponderal index (a measurement of thinness) and relevant placental parameters using the following formulas [22]: Ponderal index = birth weight (g)/birth length (cm^3) × 100; placental surface area (cm^2) = length × width × π/4; placental volume (cm^3) = surface area × thickness; and placental density (g/cm^3) = weight/volume.

2.3. Definitions

AGA was defined as a gestational age-specific birth weight between the 10th and 90th percentiles, according to a local population-based birth weight reference [20]. SGA and LGA were gestational age-specific birth weights below the 10th and above the 90th percentiles, respectively.

2.4. Assays

Cord blood samples were collected from the newborns immediately after delivery; the umbilical cord vein was double clamped by trained nurses in the delivery room before separation of the placenta to obtain a fetal blood sample. Blood samples were stored at 4 °C for up to 24 h until centrifugation. Serum samples were collected after centrifugation and were stored at −80 °C until further analysis. CTRP3 levels in cord blood were measured using a commercial enzyme immunoassay kit according to the manufacturer's instructions. Both the inter-assay and intra-assay average CVs were <8.5%. The minimum detectable level is 10 ng/ml. The linear range of the assay is 7.8–1000 ng/ml. No sample has CTRP3 levels outside the linear range.

2.5. Statistical analyses

We compared the basic characteristics between the SGA, AGA, and LGA groups using the χ^2 test for categorical variables and analysis of variance (ANOVA; with the Kruskal Wallis test where appropriate) for continuous variables. The distribution of the CTRP3 levels in the SGA, AGA, and LGA groups was described using a box plot. The normality of distribution of CTRP3 levels was examined using Kolmogorov–Smirnov Test. The result showed that CTRP3 were normally distributed in SGA and LGA groups (P values > 0.05), while distribution in AGA group was slightly skewed (P values = 0.03). In line with previous studies [14,23], we used analysis of variance (ANOVA) to explore the difference of CTRP3 levels among SGA, AGA and LGA groups and performed post hoc comparisons using the Fisher's least significant difference test. We used partial correlation coefficients (r), controlling for maternal age, parity and infant sex, to assess the correlations between CTRP3 levels and fetal growth parameters. Finally, multiple linear regression analysis was performed to examine the relationship between CTRP3 levels (dependent variable) and fetal growth status (LGA vs AGA), adjusted for maternal age, parity, and infant sex. Statistical significance was set at P < 0.05, and all statistical analyses were performed using SPSS software (ver. 17.0, SPSS Inc.).

3. Results

We enrolled 126 singleton live births (SGA, n = 34; AGA, n = 60; LGA, n = 32). Table 1 shows the characteristics of the mothers and newborns. As expected, LGA newborns had the highest birth weight and length z scores, while SGA newborns had the lowest levels (P < 0.05). Mothers of LGA newborns were less likely to have a vaginal delivery. No significant differences in infant sex, gestational age, maternal age, and parity were found among the SGA, AGA, and LGA groups (Table 1).

The median (25th–75th percentile) CTRP3 levels in the SGA, AGA, and LGA groups were 297.2 ng/ml (236.4–360.2 ng/ml), 297.5 ng/ml (261.0–369.9 ng/ml), and 368.6 ng/ml (298.5–507.1 ng/ml), respectively (P = 0.01). The post hoc comparison analysis revealed that the CTRP3 levels in the LGA group were significantly higher than those in the SGA and AGA groups (both, P ≤ 0.01). There was no significant difference in CTRP3 levels between the AGA and SGA groups (Fig. 1). In the multiple linear regression analysis, the LGA infants had a 63-ng/ml higher CTRP3 levels than AGA infants (P = 0.01), after adjusting for maternal age, parity, and infant sex.

The correlations between the CTRP3 concentrations and the fetal growth parameters are shown in Table 2. After adjustment for maternal age, parity, and infant sex, CTRP3 levels were positively correlated with birth weight z scores (r = 0.25, P < 0.01), Ponderal index (r = 0.28, P < 0.01), and placental weight (r = 0.20, P = 0.03) in the total study population. When we stratified the participants according to SGA, AGA, and LGA status, CTRP3 levels were negatively correlated with the birth length z scores (r = −0.39, P = 0.03) and were positively correlated with the Ponderal index only in the SGA group (r = 0.43, P = 0.02). Furthermore, no significant correlations were observed between the CTRP3 levels and gestational duration, placental length, placental width, placental thickness, placental surface area, placental volume, or placental density in any of the groups (Table 2). The CTRP3 levels were not significantly different between the boys and girls (median [25th–75th percentiles]: 314.62 [270.59–483.73] vs. 299.31 [254.62–365.72], P = 0.11).

4. Discussion

Here, we report that CTRP3 is present in cord blood. Moreover, CTRP3 concentrations were positively correlated with birth weight z scores, the Ponderal index, and placental weight and were significantly higher in the LGA group than in the AGA group.

The source of CTRP3 in cord blood is currently unknown. CTRP3 is ubiquitously expressed in adipose tissue, cartilage, fibroblasts, chondrocytes, vascular smooth muscle cells, and monocytic cells and is found in various human tissues [10,24–26]. It is conceivable that fetal tissue is one of the sources of CTRP3 in cord blood. Although CTRP3 is also expressed in the placenta, the amount is limited; the relatively high molecular mass of CTRP3 (30 kDa) [25] suggests that simple transport of CTRP3 across the placenta is not plausible.

The average circulating CTRP3 concentrations in previous reports were 333 ng/ml in healthy Korean adults [13], 351 ng/ml in healthy British women [23], and 416 ng/ml (measured using enzyme-linked immunosorbent assay) in Chinese women [17]. The CTRP3 level in cord blood in the present study was 340 ng/ml, which is similar to the levels found previously in adults. Collectively, these data indicate that fetal synthesis and secretion might be the main sources of CTPR3 in cord blood; further studies are needed to verify this hypothesis.

There has also been increased interest in elucidating the function of CTRP3 [14,27–29]. It can inhibit the binding of LPS to its receptor (TLR4/MD-2) and subsequently inhibit the proinflammatory process in the adipose tissue involved in obesity and type 2 diabetes mellitus [12]. By decreasing inflammation and ameliorating insulin signaling transduction, CTPR3 can increase the insulin sensitivity of insulin-resistant adipocytes [30]. CTRP3 can also increase the expression and secretion of adiponectin in murine adipocytes; adiponectin has insulin-