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Full Length Research Paper

Correlation between maternal and cord blood leptin and fetal growth

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Leptin is a protein secreted mainly by the adipocyte in proportion to fat mass. The serum leptin concentration reflects the amount of adipose tissue in the body and has potential role on the fat deposition in the fetus. In the present study, we investigated whether umbilical and maternal serum leptin concentrations correlate with fetal growth. In addition, we determined the relationship between leptin concentration in the maternal and cord blood. We studied 100 newborn infants (48 female and 52 male; gestational age, 34 - 40 weeks) and their mothers at Alzahra hospital in Tabriz city. Serum leptin concentrations were measured by ELISA and linear regression analysis was used to evaluate correlation. In the results, there was no significant correlation between umbilical and maternal leptin concentrations (r = 0.011; p = 0.459) in all study groups. There was a correlation between umbilical leptin concentration and birth weight of newborns (r = 0.278; p = 0.003) and correlation with body mass index (BMI) of the newborns (r = 0.249; p = 0.006). Maternal leptin concentrations correlated with maternal weight and BMI (r = 0.277; p = 0.003, r = 0.290; p = 0.002, respectively). There was no correlation between maternal leptin concentrations and birth weight (r = - 0.162; p = 0.054) and with BMI of the newborns (r = - 0.158; p = 0.058). There was gender difference in leptin concentrations in the newborns (r = 0.331; p = 0.00025) with greater level in females. In conclusion, we have shown that the association between umbilical serum leptin and birth weight in this and other studies suggests a pivotal role of fetal leptin in regulating fetal growth and development.

Key words: Leptin, Cord blood, Fetal Growth, Maternal.

INTRODUCTION

Leptin is 16 kDa protein encoded by the *ob* gene and produced by adipocytes (Halaas et al., 1995). The concentration of serum leptin is high in obese adults and low in lean adults (Maffei et al., 1995; Considine et al., 1996). Leptin signals the amount of fat stored in the body to the brain and affects food intake, energy expenditure, and thermogenesis to maintain a constant amount of stored body fat (Hassink et al., 1996). However, the physiological role of leptin in growing children has not been clarified.

Modeling of human leptin (Green et al., 1995) indicate that the molecule is a globular protein with a tertiary structure similar to that of helical cytokines, which include IL -2 and growth hormone. The long form of the leptin receptor functions similarly to cytokine receptors and has been detected in the human lung, kidney, liver and skeletal muscle, as well as the heart, placenta, spleen,

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Table 1. Clinical and Para clinical features of 100 infants were included in the study.

Parameter	Value
Mean gestational age (week)	38.5±1.60
Mean birth weight (gr)	3133.7±467.4
Height (cm)	48.34±2.15
Head circumference (cm)	34.69±1.25
BMI (kg/m^2)	13.4±1.83
Sex	%52 (male), %48 (Female)
Mean maternal age (years)	25.36±4.85
Cesarean section	30.9%
Neonatal gestational age range (week)	34-40 weeks
Neonatal birthweight range (g)	2020-4920 g
Neonate leptin levels range (ng/ml)	11.5± 8.33 ng/ml
Mothers leptin levels (ng/ml)	25.45±17.59 ng/ml
Mean 5-min APGAR Scores	8.88±0.65

thymus, prostate, testis, ovary, small intestine, and colon (Green et al. 1995). The finding of leptin receptors in numerous tissues supports the hypothesis that leptin is important for growth and development (Schubring et al., 1999; Schubring et al., 1997).

A series of epidemiological, clinical and experimental studies have shown that there are associations between the fetal and neonatal nutritional environment and the amount and distribution of adipose tissue in adult life. The potential impact of the prenatal nutritional experience on the development of the endocrine and neuroendocrine systems is to regulate energy balance, with a particular emphasis on the role of the adipocyte-derived hormone, leptin. In the rodent, leptin derived from the mother may exert an important influence on the development of the appetite regulatory neural network and on the subsequent regulation of leptin synthesis and the risk for obesity in the offspring. In species such as the human and sheep, there is also recent evidence that the synthesis and secretion of adipocyte-derived hormones, such as leptin, are regulated in fetal life (Bouret et al., 2004; Kratzsch et al., 2005).

The role of leptin among the complex network of factors controlling fetal growth is incompletely understood. Leptin is positively associated with birth weight, but the mechanism(s) underlying this association remains unknown. This association may reflect either a simple relationship with adipose tissue mass or an active role for leptin in fetal growth. Fat deposition in the fetus in the third trimester increases markedly. The mechanism of adiposity and physiological roles of leptin in the fetus have yet to be elucidated. In the present study, whether or not the concentration of serum leptin correlates with growth *in utero* was investigated.

A majority of leptin research in humans has so far focused on adults. In a recent preliminary report, leptin was detectable in cord blood at birth, and it correlated with birth weight (Schubring et al., 1999; Schubring et al., 1999). The present study was undertaken to examine further how different intrauterine growth patterns relate to leptin secretion *in utero.* In addition, no data exists about potential roles for leptin during gestation. In fact, during late pregnancy, leptin could be one of the links between the neuroendocrine system and the adipose tissue, which expands during pregnancy. Therefore, we tested whether leptin is present in human cord blood at birth. In addition, we measured leptin in maternal serum at term and analyzed whether there are correlations between maternal and cord leptin concentrations and growth parameters in fetus at birth.

MATERIALS AND METHODS

One hundred healthy mothers who gave birth to healthy newborns at Alzahra University Teaching Hospital from December 2005 were randomly enrolled in the study. Informed consent was obtained from all mothers. Approval of the study protocol by the Ethical Committee of Tabriz University had been taken before the study started. Clinical and auxological data (Table 1) were obtained by one investigator and recorded using a standardized data sheet. After that 5 ml maternal and 5 ml umbilical vein blood samples were obtained directly after birth.

Neonates with dismorphic features, major congenital malformations, intrauterine infections, organic disorders, or chromosomal disorders were excluded. Mother who had diabetes mellitus (MD) (gestational DM, non insulin-dependent DM, insulin-dependent DM) was excluded. The infants were divided into term (n = 80) and preterm groups (n = 20). Infants were divided into 3 subgroups: birth weight appropriate for gestational age (AGA) (n = 96), birth weight large for gestational age (LGA) (n = 2), and birth weight small for gestational age (SGA) (n =2) according to the Lubchenco chart. Gestational ages were determined from the last menstrual period of the mother and confirmed by antenatal ultrasonic measurement of the biparietal diameter. The Ponderal Index was used for nutritional assessment of the neonate. The Ponderal Index was calculated as follows: Ponderal Index = body weight (g)/[body length (cm)]^3 x 100. Placental weight directly after birth was measured.

Table 2. Correlation between the leptin concentration in cord blood with fetal growth factors.

Parameter	r	P - Value
Maternal age	-0.131	0.098
*Gestational age (sonography)	0.2	0.023*
Gestational age (LMP)	0.144	0.07
Increments of maternal weight	0.098	0.169
Maternal BMI	0.056	0.290
Maternal weight	0.093	0.180
Maternal height	0.099	0.162
Neonate Birth weight	0.278	0.003*
*Neonate Birth height	0.190	0.029*
*Neonate gender	0.331	<0.00025*
Birth kind	0.069	0.255
Head circumference	0.037	0.356
Chest	0.085	0.199
Belly	-0.006	0.476
Arm index	-0.061	0.274
APGAR score	0.070	0.244
*Placental weight	0.574	P<0.00025*
Ponderal Index	0.088	0.193
*Neonate fat mass percent	0.178	0.038*
*Neonate – BMI	0.249	0.006*
Maternal leptin	0.011	0.459

r : Correlation; *: Significant correlation.

Blood sampling and examination of serum concentration of leptin

A blood sample from the umbilical vein was obtained after double clamping of the umbilical cord immediately after birth. Venous blood of mothers were obtained after delivery from antecubital vein for study of serum leptin levels and were stored at 4^{o}C for a maximum of 12 h. They were then centrifuged, and serum was aliquot and stored at -70^{o}C until assayed. The serum leptin concentration was determined by DRG Instruments GmbH**,** DRG diagnostic, Marburg, Germany, 2395. The coefficient values of inter- and intraassays were 4.2 and 3.8%, respectively. The detection limit was 0.5 ng/mL.

Statistical analysis

Differences between groups were tested using the Mann-Whitney test for unrelated samples and the Wilcoxon test for paired samples. Linear regression analysis was used to evaluate correlation. Variance analysis was used to determine significant differences among the groups and P<0.05 considered significant.

RESULTS

100 infants were included in the study (mean gestational age, 38.5 ± 1.60 weeks). Mean birth weight was 3133.7 ± 467.4 g, height was 48.34 ± 2.15 cm, head circumference was 34.69 ± 1.25 cm, and body mass index (BMI) was 13.4 ±1.83 kg/m^2. Fifthy-two percent of studied subjects were male. Mean maternal age was 25.36 ± 4.85 yr, and cesarean section was made of delivery in 30.9% of mothers. The clinical data were summarized in Table 1. The neonatal gestational ages ranged from 34 to 40 weeks with neonatal birth weights ranging from 2020 g to 4920 g. Serum leptin levels were measured in the 100 mother/neonate pairs, and all samples had detectable leptin levels. In the neonates, leptin cord blood levels ranged from 11.50 ± 8.33 0 ng/ml. For the mothers, the range was 25.45±17.59 ng/ml. The mean, 5 min Apgar scores was 8.88 ± 0.65 in newborns.

There was no meaningful difference in maternal serum leptin concentrations delivering female or male infants (r = 0.057; P= 0.288*),* but we found significant correlation between cord leptin concentration and gender of infants (r = 0.331; P<0.00025*)*, so female infants had greater level of cord blood leptin (9.49 ± 8.23 ng/ml in males versus 13.67±7.96 ng/ml in females). Cord blood leptin concentrations correlated closely with birth weights (r = 0.278; P = 0.003), with height ((r = 0.19; P < 0.029) , with placental weight (r = 0.574; *P* = 0.00025).

Mean leptin concentrations in maternal serum at birth were 25.45± 17.59 ng/ml (range=2.9-89; median=19.9). Although, maternal leptin serum levels were higher than cord blood levels, there was no significant correlation between them (r = 0.011; p = 0.459). There was a significant correlation between leptin levels in umbilical vein and birth weight of the neonates (r = 0. 278, P < 0.003). Maternal BMI at the beginning of pregnancy and maternal body weights at the end of pregnancy were correlated with leptin levels in maternal serum at term (r = 0.290, p = 0.002 and r = 0.277; p = 0.003). Placental weight correlated inversely with leptin levels in maternal serum at birth (r = -0.084, P = 0.202) although this correlated with leptin level in cord blood (r = 0.574; p = 0.00025).

When stepwise regression analysis and ANOVA was used to determine whether placental weight or birth weight contributed more to the relation between leptin levels and auxological data, both placental weight and of birth weight remained dependent on each other. A similar and dependent contribution of placental weight and birth weight in respect to leptin concentrations was suggested by comparable P values in the ANOVA (P < 0.003) for both placental weight and birth weight. There was no relation between leptin levels in cord blood and APGAR score at 5 min after delivery (r = 0.070; p = 0.244) (Table 2). There were no significant correlations between maternal serum leptin levels and neonatal birth weights (r = -0.162, p = 0.054) but there was correlation with neonatal gestational ages at birth with LMP method (r = -0.211, p= 0.0003) and sonography (r = 0.211 p=0.018) (Table 3). Cord blood leptin levels were correlated with neither maternal body weights nor maternal body mass index at birth (r = 0.093, p = 0.180, r = 0.056, p = 0.290; respectively).

The clinical data are summarized in Table 1. There was no correlation between leptin levels in maternal serum

Table 3. Correlation between the leptin concentrations in maternal blood with fetal growth factors.

Parameter	r	P - Value
Maternal age	-0.94	0.177
*Gestational age (sonography)	-0.211	0.018*
Gestational age (LMP)	-0.270	0.003*
Increments of maternal weight (kg)	0.152	0.067
Maternal BMI	0.290	0.002*
Maternal weight	0.277	0.003*
Maternal height	-0.022	0.414
Neonate Birth weight	-0.162	0.054
*Neonate Birth height	-0.054	0.297
*Neonate gender	-0.057	0.288
Birth kind	0.119	0.127
Head circumference	-0.180	0.036*
Chest	-0.133	0.093
Belly	-0.175	0.041*
Arm index	-0.101	0.158
APGAR score	-0.62	0.270
*Placental weight	-0.084	0.202
Ponderal Index	-0.164	0.052
* Neonate fat mass percent	-0.126	0.105
* Neonate –BMI	-0.158	0.058
Maternal leptin	0.011	0.459

r : Correlation; *: Significant correlation.

and umbilical cord serum (r = 0.11, p = 0.459). We observed highly significant correlations between umbilical serum leptin levels and both neonatal birth weights (r = 0.278, p<0.003) and gestational ages at birth (r = 0.2, p = 0.023) (Table 2). We also found correlation between cord leptin and neonatal fat mass (r = 0.178, p = 0.038).

DISCUSSION

Our study showed that leptin levels were less in umbilical cord serum than in maternal serum and this study suggests that leptin of fetus is produced by itself or by placenta. In intrauterine fetal growth, Hassink et al. (1997) reported the synthesis of leptin in the placenta, and that the leptin concentration in cord blood correlates with newborn anthropometry. In addition, umbilical serum leptin levels were found to correlate positively with neonatal birth weights. Therefore, fetal leptin levels may be involved in fetal development during late pregnancy. These results suggest that rapidly growing fetal adipose tissue is a major contributory factor. It has been shown that, after 32 weeks of gestation, fetal growth occurs via cellular hypertrophy, and that most fetal fat and glycogen deposition takes place during this phase (Jaquet et al., 1998; Hoggard et al., 2001; Schubring et al. 1998). Given the above observations, we hypothesize that during late gestation, fetal leptin may stimulate fetal growth. The presence of leptin in human cord blood has been reported by several investigators (Schubring et al., 1999). Koistinen et al. (1997) found a positive association of leptin concentration in cord blood with intrauterine growth. Matsuda et al. (1997) found serum leptin concentration in cord blood to be correlated positively with BW (r = 0.555; P < 0.001) in the study of 82 newborns ranging in gestational age from 36 weeks to 42 weeks and in BW from 2306 to 4128 g (Matsuda et al., 1997). A similar correlation (r = 0.57; P = 0.03) between leptin concentration in cord blood and BW was reported by Schubring et al. (1997) who noted a positive association between cord blood leptin concentration and placental weight (r = .50; P < .01). Our study showed positive correlation between cord blood leptin concentration and placental weight (r = 0.574; p = 0.00025) too.

Umbilical serum leptin levels were also found to correlate positively with neonatal gestational ages at birth. This suggests that the increased umbilical serum leptin levels associated with advancing gestational age are a consequence of increased fetal body weight. Also we observed that maternal serum leptin levels were correlated with maternal body weights, body mass index at delivery and gestational age but that there was no correlation with neonatal birth weights. It seems that maternal serum leptin levels may only be a marker for maternal fat mass, but do not play a major role in fetal growth. In contrast, BMI at delivery and maternal weight did not correlate with leptin levels in maternal serum in Schubring et al. (1997). It is unclear what specific regulators of leptin levels might be effective during gestation and at term. These data might simply reflect the poor correlation of BMI and weight measurements with fat tissue expansion during pregnancy. However, a more perfect study design may be warranted, which would allow us to be more conclusive.

Hormonal regulation of leptin levels in the fetus and neonate might be different from the endocrine modulation of leptin levels seen throughout adult life; whereas insulin and glucocorticoids (Sinha et al., 1996; Rentsch and Chiesi, 1996) are thought to modulate leptin levels throughout adult life, this might not be entirely relevant for the fetus and neonate. There was no sex difference of leptin levels in cord blood at term reported by Shekhawat et al. (1998) and Shekhawat et al. (2000). This is in contrast to the situation in the adult where consistently higher levels of leptin are found in serum from females than from males (Considine et al., 1996; MacDougald et al., 1995). One possible explanation for the absence of a gender difference of leptin levels at birth might be that the body fat mass of a neonate is not gender specific.

In summary, our study showed that leptin levels were higher in maternal serum than umbilical cord serum (11.5±8.33 vs 25.45±17.59). In addition, umbilical serum leptin levels were found to correlate positively with neonatal birth weights. Therefore, fetal leptin levels may be involved in fetal development during late pregnancy.

Our data indicate that birth weight is dependently associated with leptin concentrations in cord blood of newborns. This suggests that leptin may be involved in as-yet-unknown mechanisms that regulate fetal growth. Additional studies will likely lead to an understanding of the mechanism(s) by which leptin may regulate fetal growth.

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