

# Comparison of Umbilical Cord and Maternal Serum Levels of IGF-1, Leptin and Cortisol in Appropriate for Gestational Age and Small for Gestational Age Neonates

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We aim to compare Insulin like Growth Factor (IGF-1), leptin, and cortisol concentrations in maternal and umbilical cord vein and to investigate the relationship between these values and fetal growth in appropriate for gestational age (AGA) and small for gestational age (SGA) neonates.

**Materials and Methods:** In a case-control study, maternal and umbilical cord venous samples were collected from 25 SGA neonates and 25 AGA neonates in the obstetric ward at Hafez Hospital, Shiraz, Iran, between 2004 and 2005. Serum levels of IGF-1, leptin, and cortisol were measured by specific radioimmunoassay using commercial kits.

**Results:** Mean maternal age was  $25.38 \pm 5.22$  years (range 17-38 yr) and mean gestational age was  $37.92 \pm 1.79$  weeks (range 34-41 weeks). Mean concentration of leptin and IGF-1 in cord blood was lower in SGA as compared with AGA neonates ( $p < 0.012$  and  $p < 0.001$ , respectively). Maternal serum concentration of IGF-1 and cortisol were lower in SGA neonates ( $p < 0.032$  and  $p < 0.011$ , respectively), however there was no significant difference in the concentration of maternal leptin levels between the two groups. A correlation was observed between the head circumference of neonate and maternal cortisol levels in the SGA group ( $p < 0.01$ ).

**Conclusion:** Low IGF-1 and leptin concentrations in cord blood and low maternal serum concentration of IGF-1 and cortisol are associated with growth retardation in SGA as compared to AGA neonates. Also maternal cortisol level plays an important role in intrauterine brain development of SGA neonates.

**Key Words:** Leptin, Cortisol, IGF-1, Intrauterine growth retardation

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## Introduction

The term intrauterine growth retardation (IUGR) or small for gestational age (SGA) is assigned to newborns with birth weight and/or birth length below the 10th percentile for their gestational age (birth weight  $< 2500$  gr) and the term AGA is assigned to newborns with birth weights of over 2500 gr (above 10th percentile for their gestational age).<sup>1,24</sup> Fetal growth restriction is a serious complication of pregnancy, leading to an increased risk of prenatal hypoxia, preterm delivery, and fetal demise.<sup>2</sup>

There is increasing evidence that the foundations of life-long health are, in part, laid in the uterus. Recent studies show that in IUGR infants, the long term health risk continues,<sup>2</sup> and therefore understanding the mechanisms controlling fetal growth and causes of fetal growth retardation is of extreme importance, since this facilitates therapeutic options. It seems likely that IGF-1 and leptin play central roles in controlling fetal growth.<sup>2-6</sup>

In the present study, we aimed to compare the IGF-1, leptin, and cortisol concentrations of maternal and umbilical cord venous samples between appropriate for gestational age (AGA) and small for gestational age (SGA) neonates, to determine any relationship between fetal growth and serum level of IGF-1, leptin & cortisol. Could these serum markers be used to predict birth weight, head circumference (HC) and perinatal complication of small for gestational age infants?

## Materials and Methods

Between 2004 and 2005, in a nested case-control study, maternal and umbilical cord venous samples were collected from 25 SGA neonates (birth weight <2500 gr) and 25 AGA neonates (birth weight  $\geq$ 2500 gr) in the obstetric ward at Hafez Hospital, Shiraz, Iran. We matched the groups based on maternal age, since there are more complications of pregnancy in the mother and fetus, below the age of 18 and over the age of 40 years and this could affect our results. Neonates with congenital malformation chromosomal abnormalities, metabolic disease and neonatal infections were excluded.<sup>2-4</sup>

Data was obtained from antenatal care chart evaluations and pediatric examination records at times of delivery and discharge from hospital. All pregnant women included in this study had normal results for 50 gram glucose challenge tests during the 3rd trimester (glucose cut off level  $\geq$ 140 mg% after 1 hr); smoking status during pregnancy was noted from the antenatal care records and on admission for delivery. Women with hypertension and preeclampsia were excluded from this study. Samples obtained from mothers with a calculated body mass index (BMI)  $>$ 29 were excluded.<sup>2-4</sup> Maternal and neonatal data were collected using ongoing medical records.

Maternal blood samples were obtained from the antecubital vein, at the time of delivery. The umbilical vein samples were taken immediately following clamping of the cord, before separation of the placenta; samples were separated

by centrifugation, and the serum was stored at -20°C for upto 4 months.

Serum levels of IGF-1, leptin and cortisol were measured at the Shiraz University Endocrine Research Center by a two-site radioimmunoassay using commercial kits (IRMA A 15729 Immunotec, KA C2282/KA C2281 and RIAIM 1841 Immunotec, Gunma, Japan); for this assay, detection limit is 0.031  $\mu$ gr/dl and within and between assay coefficients of variation are less than 8.4%.

All anthropometric measurements were taken by a trained nurse using standard methods.<sup>23</sup> Weights were measured using the same scale (Seca Company, Germany).

Statistical analysis was performed by descriptive statistics (frequency analysis and mean values $\pm$ SD), student t-test for mean comparisons and Fisher exact test for multiple comparisons. Statistical significance was set at  $p < 0.05$  for all tests. Correlations were studied using the Pearson correlation coefficient. A Pearson correlation coefficient  $>$ 0.6 was calculated to be significant at the 0.05 level for our sample size order. This study was approved by our university ethics committee. Informed consent was obtained from the mothers.

## Results

Characteristics of 50 newborns (25 SGA and 25 AGA) with mean gestational age of 37.92 $\pm$ 1.79 weeks (range 34-41) and mean maternal age of 25.38 $\pm$ 5.22 years (range 17-38) are depicted in table 1.

Mean concentration of leptin and IGF-1 in cord blood (CB) was lower in SGA compared with AGA neonates ( $p < 0.012$  and  $p < 0.001$ , respectively) (Table 2).

**Table 1. Characteristics of SGA and AGA neonates**

Characteristics	SGA	AGA
Head circumference (cm)	31.72±1.69*	34.56±1.38
Birth weight (gm)	2060±301	3200±394
Height (cm)	44.8±2.32	48.56±2.57

\* Mean±SD

**Table 2. Umbilical cord leptin, IGF-1, and cortisol levels**

(µg/dL)	SGA	AGA	P-value
Leptin	14.1±6.9*	22.7±14.2	<0.012
IGF-1	41.3±28.8	72.2±28.1	<0.001
Cortisol	11.5±9.3	17.5±12.6	0.062

\* Mean±SD

Maternal serum concentrations of IGF-1 and cortisol were lower in SGA neonates ( $p<0.032$  and  $p<0.011$ , respectively), but there was no significant difference in the concentration of maternal leptin levels between the two groups (Table 3).

**Table 3. Maternal serum leptin, IGF-1, and cortisol levels**

(µg/dL)	SGA	AGA	P-value
Leptin	33.72±25.25*	36.76±18.77	0.631
IGF-1	221±112	308.89±162	<0.032
Cortisol	42.08±22.01	57.82±20.19	<0.011

\* Mean±SD

There was no correlation between sex or Lt, and serum level of leptin, IGF-1, and cortisol in maternal and umbilical cord venous samples.

Birth weight (BW) was found to be correlated with IGF-1 ( $r=0.32$ ,  $p<0.05$ ) and cortisol ( $r=0.43$ ,  $p<0.01$ ) in maternal serum, and with IGF-1 ( $r=0.31$ ,  $p<0.05$ ) and leptin ( $r=0.30$ ,  $p<0.05$ ) in CB sample. HC was correlated with maternal serum cortisol level ( $r=0.31$ ,  $p<0.05$ ). There was no correlation between HC and serum leptin and IGF-1.

Following classification into the SGA and AGA groups, statistical calculation was

repeated. It showed that in both groups, BW was correlated with IGF-1 ( $p<0.05$ ,  $p<0.01$ ) and cortisol ( $p<0.02$ ,  $p<0.01$ ) in maternal serum, and with IGF-1 and leptin in cord blood sample ( $p<0.01$ ,  $p<0.01$ ). There was no relationship between HC of neonates and maternal cortisol level in AGA group ( $p>0.05$ ), but in the SGA group, the HC of neonates was correlated with maternal cortisol level ( $p<0.01$ ).

In all newborns CB IGF-1 had a correlation with CB leptin ( $r=0.81$ ,  $p<0.01$ ) and CB cortisol ( $r=0.49$ ,  $p<0.01$ ). In addition, CB cortisol was correlated to maternal cortisol ( $r=0.55$ ,  $p<0.01$ ) and CB IGF-1 and maternal IGF-1 levels ( $r=0.40$ ,  $p<0.01$ ) were correlated.

## Discussion

Growth control in the fetal and neonatal period is a complicated process. Insulin is the main regulator of IGF-1 in prenatal period and both of these hormones along with IGFII are key factors in controlling fetal growth.<sup>4</sup>

In the present study, we found that IGF-1 in CB and maternal serum is correlated with BW. Few reports exist of a positive correlation between IGF-1 level and birth size, supporting the idea that fetal insulin and IGF-1 levels are the primary hormones involved in the regulation of fetal growth.<sup>2-4</sup>

Zhang et al demonstrated a positive correlation between BW and CB IGF-1. In the Orbak et al study, CB IGF-1 levels were significantly lower in SGA compared with AGA neonates.<sup>7,8</sup>

As maternal IGF-1 does not cross the placenta, the mechanism of its effect on fetal growth is unclear. Perhaps it is related to changes in placental function and to improved provision of substrates to the developing fetus. Synthesis, storage, and release of leptin by the placenta are continuous processes throughout pregnancy; leptin levels increase during pregnancy and return to baseline after delivery.<sup>9-11</sup>

Conflicting results have been documented comparing leptin concentrations in the cord

blood of SGA and AGA newborns; some studies have reported lower,<sup>12-14</sup> and others, higher leptin levels in SGA, as compared with AGA newborns.<sup>15</sup> Shekhawat et al<sup>15</sup> found that SGA neonates have elevated CB leptin levels and suggested that insulin resistance, hyperinsulinemia, and elevated concentrations of endogenous corticosteroid hormones in SGA infants are responsible for the elevated leptin concentration.

In another study,<sup>16</sup> CB leptin levels were correlated with neonatal and placental size in the AGA, but not the SGA group. Lo et al observed that IGF-1 and leptin are significantly correlated with BW, leptin and HC in preterm and term neonates.<sup>17</sup>

A role for leptin in pregnancy was later suggested by the findings that gestational plasma levels dosing are greater than in non gravid individuals and that leptin is synthesized within the fetoplacental unit.<sup>18</sup>

Observational studies have established that abnormal leptin levels affect several pathological stages of pregnancy in association with alterations of fetal growth. For example, an overproduction of leptin by the placenta in pregnancy with DM or HTN is associated with maternal hyperleptinemia. Evidence is also accumulating that umbilical leptin levels can be viewed as a biomarker of fetal adiposity.<sup>18</sup>

Hauguel-de Mouzon et al in their study of leptin in pregnancy have contributed largely to the insight into the mechanisms of leptin action, both as hormones & as cytokines.<sup>18</sup>

In line with previous studies, maternal leptin concentrations of SGA neonates in our study did not significantly differ from those of AGA neonates, but CB IGF-1 and leptin were lower in SGA neonates as compared with AGA neonates.

The role of fetal cortisol in intrauterine growth retardation (IUGR) is unclear. In the current study, maternal serum cortisol was lower in SGA group than AGA. According to Chernukha et al,<sup>19</sup> in the course of vaginal delivery, maternal cortisol level increases significantly, the rise being higher in

parturients with growth retarded fetuses. Economides et al<sup>20</sup> reported that in the SGA fetuses, plasma cortisol was higher and ACTH was lower than in AGA fetuses. Christian Plank et al found no alterations in systemic cortisol-cortisone conversion either in short children born SGA or in SGA rats. However local modifications of the 11 B-HSD system may be possible.<sup>21</sup>

Siler-Khodr et al also demonstrated that ethnicity is a significant factor affecting corticotropin-releasing hormone concentrations at midgestation in the Hispanic and white populations. The use of ethnicity-specific medians to estimate the ethnicity-specific MoM for the corticotropin-releasing hormone concentrations may enhance the predictive value of midgestational maternal corticotropin-releasing hormone as a screening parameter for the prediction of preterm birth.<sup>22</sup>

In addition to all the above, the number of the neonates in this study, a limitation, could have influenced the results.

Considering the controversy regarding a relation between maternal and fetal cortisol levels and fetal growth, further investigations to determine the precise effect of cortisol on fetal growth are recommended.

Low IGF-1 and leptin concentrations in cord blood and low maternal serum concentration of IGF-1 and cortisol are associated with growth retardation in SGA as compared to AGA neonates. Use of these serum markers may predict whether newborns may be SGA or not, since we do not have any scale to predict birth weight by these serum markers, yet. In addition, cortisol levels of mothers of SGA are correlated with HC of neonates, indicating that maternal cortisol level may play an important role in intrauterine brain development (evaluated by head circumference of the neonates). Much research is needed to determine how levels of maternal cortisol and IGF-1 can be increased to prevent SGA and improve intrauterine brain development.

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