Cord blood level of insulin-like growth factor-1 and IGF binding protein-3 in monochorionic twins

Ru-Jeng Tenga,*, Tzong-Jin Wua, Fon-Jou Hsiehb

a Division of Neonatology, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI 53226, USA
b Department of Obstetrics and Gynecology, National Taiwan University, Taipei, Taiwan

Received 19 August 2012; received in revised form 27 November 2012; accepted 26 December 2012

KEYWORDS
insulin-like growth factor (IGF); insulin-like growth factor binding protein (IGFBP); intrauterine growth; monochorionic twin

Background/Purpose: Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are known to modulate fetal growth but their role in intrauterine growth of monochorionic twins (MCT) has not been studied.

Methods: Cord venous blood was collected directly after birth. IGF-1 and IGFBP-3 in the cord venous blood were quantified by radioimmunoassay. Birth weights (BWs) were obtained electronically. Placentas were examined for chorionicity.

Results: Cord blood was collected in 37 pairs of MCT (15 pairs were males). BWs ranged from 564 to 3240 g, and gestational ages (GAs) were between 24 weeks and 39 weeks. There was a correlation between BW and cord venous blood IGFBP-3 concentration ($r = 0.28$, $p = 0.015$), but not between BW and cord venous blood IGF-1 level. There was no difference in IGF-1 between the heavier twins ($30.8 \pm 61.8 \text{ ng/mL}$) and lighter twins ($33.2 \pm 63.7 \text{ ng/mL}$), but a trend ($p = 0.096$) of higher IGFBP-3 level was demonstrated in heavier twins ($3.14 \pm 1.23 \text{ mg/mL}$) than in lighter twins ($2.71 \pm 1.19 \text{ mg/mL}$). The IGFBP-3 levels were higher ($p = 0.042$) in female twins ($3.20 \pm 1.33 \text{ mg/mL}$) than in male twins ($2.64 \pm 1.04 \text{ mg/mL}$). The IGF-1 level of the heavier twins correlated significantly to their lighter co-twin ($r = 0.73$, $p < 0.001$).

Conclusion: Our data showed that cord venous blood IGF-1 level might be controlled mainly by genetic factors. IGFBP-3 might play an important role in fetal growth.

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Introduction

Insulin-like growth factors (IGFs) are a family of polypeptides with growth promoting and insulin-like activities, separated into two main classes, IGF-1 and IGF-2, by their molecular weight and biological properties. Intrauterine fetal growth is believed to be controlled by both the IGF axis and maternal factors. The actions of the IGFs are modulated by insulin-like growth factor binding proteins (IGFBPs). Six binding proteins, IGFBP-1 to IGFBP-6, have been characterized to date, of which IGFBP-3 is the most important in human blood. There were several studies demonstrating that serum IGF-1 levels correlated positively with fetal weight, length, and placental weight. Because of the wide variation of IGF-1 in cord blood, and complex interplay among IGFs and IGFBPs, contradictory results were seen from previous studies. Study in full-term newborn infants demonstrated a good correlation between IGFBP-3 level and birth weight (BW). In an attempt to confirm the relationship between cord IGF-1 and IGFBP-3 levels and fetal growth, we selected monochorionic twins as our study subjects due to their identical genetic makeup.

Subjects and methods

A total of 37 pairs of monochorionic twins, delivered in the National Taiwan University Hospital, were enrolled prospectively in our study. Informed consent was waived by the Institutional Review Board because cord blood was viewed as medical waste as long as patients’ identifiers were not reported. Cord venous blood was collected and centrifuged at 3000 rpm for 15 minutes and plasma was stored at −80°C until analysis. Monochorionicity was confirmed by placental inspection and milk injection. BW was measured to the nearest gram using electronic scales. The IGF-1 and IGFBP-3 levels were quantified by radioimmunoassay kits (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA) in triplicate. An acid–ethanol extraction method was used for sample preparation before the IGF-1 radioimmunoassay. All reagents required for the assay were prepared according to the manufacturers’ instructions. The sensitivity was 0.3 ng/mL with intra-assay variation below 3.0% and inter-assay variation below 8.4%. In the IGFBP-3 assay, the sensitivity was 0.12 μg/mL with the intra-assay variation below 8.0% and the inter-assay variation below 6.3%.

The paired Wilcoxon test was used to compare the IGF-1 and IGFBP-3 levels between heavy and light twins for their skewed distributions. The Mann-Whitney U test was used for other comparisons. To compare the proportions of small for gestational age (GA) as compared to 17 of 37 of their light co-twins [Chi-squared test 7.63, odds ratio (OR) 0.228, 95% confidence interval (CI) 0.077–0.675, p < 0.01]. The GA was similar between male twins (35 ± 3 weeks) and female twins (35 ± 2 weeks). There was no difference in BWs between male twins (2161 ± 514 g) and female twins (2198 ± 480 g). IGF-1 levels were between 11.25 ng/mL and 402.75 ng/mL, and IGFBP-3 levels were between 0.24 μg/mL and 6.30 μg/mL. No difference (p > 0.5) was detected between male and female twins in respect of body length and head circumference in our subjects.

Significant positive correlation was observed between BW and IGFBP-3 concentration (r = 0.28, p = 0.015) (Fig. 1) but such correlation was not observed between BW and IGF-1. We also failed to detect any correlation between birth length and IGF-1 level (p > 0.5). There was no difference in cord venous blood IGF-1 between heavy twins (30.8 ± 61.8 ng/mL) and light twins (33.2 ± 63.7 ng/mL) (Fig. 2), but a trend (p = 0.096) towards higher IGFBP3 levels could be demonstrated in heavy twins (3.14 ± 1.23 μg/mL) compared to light twins (2.71 ± 1.19 μg/mL) (Fig. 3). The IGFBP3 levels were significantly higher (p = 0.042) in female twins (3.20 ± 1.33 μg/mL) than in male twins (2.64 ± 1.04 μg/mL). The IGF-1 level of the heavy twins correlated significantly with their co-twins (r = 0.75, p < 0.001), but the IGFBP3 level did not (p = 0.24). After controlling for BW, sex, birth length, head circumference, and GA, there were no differences for IGF-1 (F = 1.6, p = 0.21) and IGFBP-3 (F = 0.002, p = 0.96) between small-for-GA and appropriate-for-GA twins.

Discussion

Two IGFs have been studied to date in human subjects, IGF-1 and IGF-2, and the cord blood levels have been shown to correlate with gestational age or birth weight of the newborn infants. Low IGFs have been shown in small-for-GA infants. In term infants, the cord levels of IGF-1 and IGFBP3 significantly correlate with BW, Ponderal index, and placental weight. Owing to a variety of bioassay...
techniques, and differences in the study subjects, the results have not been conclusive to date.\textsuperscript{9} Intrauterine growth retarded infants have significantly lower levels of IGF-1\textsuperscript{16,18} and IGFBP-3,\textsuperscript{19} but conflicting results have been reported.\textsuperscript{9}

The actions of the IGFs are modulated by the IGFBPs.\textsuperscript{4} IGFBP-3 is the most important one: it accounts for the majority of IGF binding, and it serves as the reservoir of IGFs.\textsuperscript{6} Decreased levels of IGFBP-3 have been shown in the cord blood of intrauterine growth retarded infants.\textsuperscript{20}

Monochorionic twins, with identical genetic makeup, are useful to study the relationship between IGFs, IGFBPs, and fetal growth. It has been shown that monozygotic twin children have high within-pair correlation of IGF-1.\textsuperscript{21} A similar result has also been shown in adult twins.\textsuperscript{21} The results of these studies suggest a genetic contribution to the inter-individual variation of the levels of IGF-1 post-natally. Although mutations in genes controlling IGF, IGFBP, and IGF receptors have been reported to be associated with intrauterine growth retardation, the results obtained from our study subjects, monochorionic twins, may eliminate most of these concerns. We demonstrated the within-pair correlation of the cord venous levels of IGF-1 in monochorionic twins. Our model removed the environmental effects on IGF-1 and IGFBP-3 and further strengthens the genetic contribution of the IGF axis in fetal growth. From our results, we believe that the correlation between BW and cord level of IGF-1 in previous reports may be a secondary effect. It is the environmental factors that determined the BW rather than IGF-1 level. Judging from the high heritability of IGF-1 in a recent twin study, we believe that IGF-1 levels are determined mainly by genetic attributes.\textsuperscript{22,23}

Our data show that the cord venous IGFBP-3 level is higher in females than in males, which is similar to twin studies performed in adults.\textsuperscript{23} However, we failed to show significant intra-twin correlation in IGFBP-3 levels. The significance of higher IGFBP-3 in female infants is unclear, because our results showed that IGFBP-3 levels were correlated with BW but there was no weight difference between male twins and female twins. Further studies may be required to clarify this issue.

In conclusion, our data suggests that the IGF-1 level in cord blood is more genetically determined, whereas IGFBP3 may play an important role in determining fetal growth. Due to the low prevalence of monochorionicity, our data fail to answer several important issues. Future collaboration among major centers may provide more information in this regard.

**Acknowledgments**

This study was supported by a grant (NSC 85-2331-B-002-328b to RJT) from the National Science Council, Republic of China. We are indebted to Dr Elaine St. John, Dr Sidhartha Tan, and Dr Ambalavanan Namasivayam for their help in editing the manuscript.

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