The Role of Umbilical Cord Blood Insulin Like Growth Factor-1 Levels in the Assessment of Bone Maturation in Term Neonates

Term Bebeklerde Kemik Matürasyonunun Belirlenmesinde Kord Kanı İnsülin-benzeri Büyüme Faktörü (IGF-1) Düzeylerinin Rolü

Selim Kurtoğlu
Prof., M.D.
Department of Paediatric Endocrinology
Erciyes University Medical Faculty
selimk@erciyes.edu.tr

Mehmet Emre Atabek
Assoc. Prof., M.D.
Department of Paediatric Endocrinology
Selçuk University Meram Medical Faculty
meatabek@hotmail.com

Özgür Pirgon
Assist. Prof., M.D.
Department of Paediatric Endocrinology
Selçuk University Meram Medical Faculty

Nihal Hatipoğlu
Specialist, M.D.
Kayseri Maternity Hospital
Division of Peadiatric Endocrinology
nihalhatipoglu@yahoo.com

Abstract
Purpose: In this study, we aimed to investigate the relation of anthropometric data and knee epiphyseal area with cord blood Insulin Like Growth Factor-1 (IGF-1) levels at birth in term neonates.
Material and Method: Twenty-six term neonates were recruited. Anthropometric measures and the knee epiphyseal area were recorded and umbilical cord blood samples were collected at birth. The knee epiphyseal area was calculated by the addition of [width x height x 3.1415/4] of the distal femoral epiphysis and proximal tibia epiphysis.
Results: Mean concentrations of IGF-1 levels of cord blood and the knee epiphyseal area were 45.2 ± 36.8 ng/ml and 29.0 ± 18.4 mm2 in term neonates, respectively. IGF-1 levels correlated significantly with knee epiphyseal area (r: 0.45, p: 0.018). In a multiple regression model, IGF-1 emerged as independent correlates for mean knee epiphyseal area in term neonates with the total variance being 18 %. Although IGF-1 level had significantly positive correlations with body surface area, birth weight, midarm circumference did not correlate with gestational age, birth length, head circumference, ponderal index and skinfold thickness.
Conclusion: Cord blood IGF-1 level is useful for assessing the bone maturation in neonates, because a changes in IGF-1 correlates with the change in epiphyseal area.

Key words: **Epiphyseal Plate; Fetal Development; Insulin-like Growth Factor-1.**

Özet
Amaç: Bu çalışmada term bebeklerde antropometrik ölçümler ve diz epifiz yüzeyi ile kord kanı IGF-1 düzeyi arasındaki ilişkiler araştırıldı.
Gereç ve Yöntem: Çalışmaya 26 term bebek alındı. Doğumda kord kanı IGF-1 düzeyi ölçüldü ve antropometrik ölçümler alınırken, diz grafisinde epifiz yüzeyi en boy ve pi/4 çarpımıyla hesaplandı.
Bulgular: Kord kanı IGF-1 düzeyi ortalaması 45,2 ± 36,8 ng/ml ve epifiz yüzeyi 29,0 ± 18,4 mm^2 bulundu. Kord dayanarak kord kanı IGF-1 düzeyinin kemik ve vücut büyümesi üzerine etkili olduğu sonucuna varıldı. Ayrıca kord serum IGF-1 düzeyi ile vücut yüzeyi, doğum ağırlığı, sol orta kol çevresi arasında pozitif korrelasyon bulunurken, gebelik haftası, doğum boyu , başçevresi ve triseps cild kıvrım kalınlığı ile korrelasyon saptanmadı.
Sonuç: Elde edilen verilere dayanarak, kord kanı IGF-1 düzeyinin kemik ve vücut büyümesi üzerine etkili faktörlerden biri olduğu sonucuna varıldı.

Anahtar Kelimeler: **Epifiz Düzlemi; Fetal Gelişme; İnsülin benzeri Büyüme Faktörü-1.**

This work has bean supported by Erciyes University Research Found.

Submitted : May 27, 2008
Revised : June 05, 2009
Accepted : July 15, 2009

Corresponding Author:
Prof. Dr. Selim Kurtoğlu
Department of Paediatric Endocrinology
Faculty of Medicine, University of Erciyes
38039 Kayseri- Turkey

Telephone: +90- 352 4374901 / 22105
E- mail: selimk@erciyes.edu.tr

Introduction

Both growth hormone and insulin-like growth factor-1 (IGF-1) have direct effects on the growth plate. Surrounding the growth plate, growth hormone also influences bone itself, connective tissue, vasculature and the bone marrow, and injections of either growth hormone or IGF-1 to hypophysectomized rats stimulates growth plate chondrocytes at all stages of differentiation. The effect of growth hormone is greater than that of IGF-1, but none of them completely restore the hypophysectomized animal's growth (1) In a current study, the data suggest that circulating IGF-1 is critical for the modeling of bone, particularly periosteal growth (2) In addition, in our recent study, we found a relationship between cord blood IGF-1 and bone mineral density in preterm neonates, suggesting that even within an unremarkable population, IGF-1 might be important to ensuring bone health (3).

In the present study, we aimed to investigate the relation of anthropometric data and knee epiphyseal area with cord blood IGF-1 level at birth in term neonates.

Material and Methods

Subjects. The samples for cord blood taken from the umbilical veins were collected from 26 term neonates dating pregnancy from the first day of the last menstrual period was used to assess gestational age. All term neonates had normal thyroid functions and normal growth hormone levels and weights appropriate for gestational age, that is, birth weight between the 10th and 90th percentiles of the normal growth chart of infants, and were free of major congenital anomalies, congestive heart failure, renal disease, or major surgical procedures. In addition, mothers of those neonates were free of cardiovascular disease, renal and liver diseases, hypertension, and diabetes. Informed consent was obtained from the parents prior to the inclusion of their children in the study. The ethics committee of the Erciyes University, Faculty of Medicine approved the study (May, 1996).

IGF-1 analysis. The nurses at the delivery room took all umbilical cord blood samples. Serum was separated and samples were frozen at -20°C prior to analysis. Serum IGF-1 concentrations were determined by a 2-site immunoradiometric assay using the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-1 interassay CV was 3.7% to 8.2%, and intra-assay CV was 1.5% to 3.4%. Assay sensitivity was 0.8 ng/mL.

Anthropometric measurements. Birth weight and birth length were obtained from each term neonate immediately after birth. Measurement of head circumference was done on day 2 to allow for resolution of edema and head molding. Birth length, midarm circumference and head circumference were measured with plastic-covered fabric measuring tapes read to the nearest mm. Based on birth weight and birth length, ponderal index [(Birth weight (g) /Birth length (cm)x100)] was calculated. Skin fold thickness was measured by Harpenden Skinfold Calipers over triceps at mid-arm. Standard deviation scores were assessed as using the 1978 Turkish Standards (4).

Epiphyseal area measurements. In order to evaluate the epiphysial cores of the neonates, their knee x-rays were taken in antero-posterior position at a suitable distance. The dimensions of epiphysis were measured on the film using calipers. Total knee epiphyseal area was obtained by the addition of right distal femoral and right proximal tibial area calculated by [width x height x 3.1415/ 4] formula (5; Figure 1). The intra-observer and the inter-observer reliability of knee epiphyseal area were determined by calculating the correlation coefficient and intra-class correlation coefficient (ICC) were calculated.

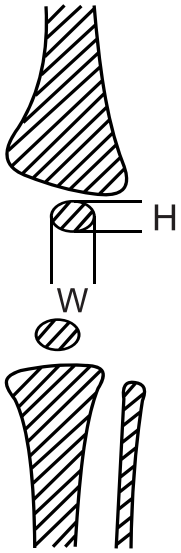

Figure 1. Calculation of the knee epiphyseal area. H: Epiphysial height; W: Epiphysial width. Total knee epiphyseal area = width (W) x height (H) x 3.1415/ 4 (for right knee)

Statistical analyses. Values are expressed as mean ± standard deviation. The Kolmogorov-Smirnov test was applied separately for boys and girls to check the normality of the variables. Differences between data were studied using the Student's t test. Pearson correlation coefficients were used to quantify relationships between variables. Multiple linear regression analysis in standard and forward stepwise selection was performed to identify independent factors affecting knee epiphyseal area and to estimate the final predictors of its variability. Statistical significance was taken as $p < 0.05$.

Results

There were 14 males and 12 females among term neonates. No difference was found between male and female neonates for anthropometric data and IGF-1 levels. Anthropometric characteristics and gestational age of term neonates are listed in Table I. The gestational age averages were 39.6 ± 0.8 wk for term neonates. Knee epiphyseal area was 29 ± 18.4 mm2 in term neonates. Pearson's r and ICC was 0.96 and 0.95 for the intra-observer reliability of knee epiphyseal area and 0.89 and 0.87 for the inter-observer reliability of knee epiphyseal area, respectively.

Table I. Characteristics of term neonates

Characteristics	
Gender (n)	
Male	14
female	12
Gestational age (week)	38.6±0.8
Birthweight (g)	3354.2±411.3
Birthlength (cm)	50.4±2.1
Herad circumference (cm)	34.6±1.3
Midarm circumference (cm)	10.3±1.2
Ponderal index (g/cm3 x 100)	2.6±0.2
Skinfold thichness (mm)	7.7±1.6
Knee epiphysial area (mm^2)	29±18.4
Body surface area (m^2)	0.3±0.05
IGF-1 (ng/mL)*	45.2±36.8

* IGF-1: Insulin like growth factor

The correlations among anthropometric characteristics and gestational age and serum IGF-1 in term neonates are shown in Table II. Cord serum levels of IGF-1 had significantly positive correlations with body surface area (r: 0.45, p: 0.018), knee epiphyseal area (r: 0.45, p: 0.018), birth weight (r: 0.42, p: 0.032) and midarm circumference (r: 0.5, p: 0.009) in term neonates. There were no significant correlations between IGF-1 levels and gestational age (r: 0.04, p: 0.82), birth length (r: 0.15, p: 0.46), head circumference (r: 0.21, p: 0.27), ponderal index (r: 0.33, p: 0.092) and skin fold thickness (r: 0.22, p: 0.28) in term neonates.

Table II. Regression analysis between cord IGF-1 and other parameters

Characteristics	R	P
Gestational age (week)	0.04	NS
Birth weight (g)	0.42	0.032
Birth length (cm)	0.15	NS
Head circumference (cm)	0.22	NS
Midarm circumference (cm)	0.5	0.009
Body surface area (m^2)	0.45	0.018
Ponderal index (g/cm^3 x100)	0.33	NS
Skinfold thickness (mm)	0.22	NS
Knee epiphyseal area (mm^2)	0.45	0.018

R: regression coefficient; p: significance level; NS: Not statistically significant ($p > 0.05$).

In a multiple regression model, we included IGF-1 and anthropometric parameters as independent variables in the model for knee epiphyseal area. IGF-1 emerged as independent correlates for mean knee epiphyseal area levels (ß= 0.43, p= 0.02) in term neonates with the total variance explained being 18 %.

Discussion

In the study, we examined the relation between IGF-1 levels and the measurement of epiphyseal area related to bone maturation. IGF-1 levels of cord blood showed a significant positive correlation with epiphyseal area in term neonates. IGF-1 is an essential factor for longitudinal bone growth, as it stimulates proliferation and differentiation of chondrocytes in the epiphyseal plate (6) IGF-1 also has potent stimulatory effects on synthesis of bone-specific proteins and osteoblastic proliferation in cell and organ cultures in vitro (7,8) Messenger RNA for IGF-1 has been demonstrated in cultures of human

osteoblasts (8). Maor et al. isolated reserve cells from a newborn mouse mandibular condyle, and showed that in vitro incubation with either growth hormone or IGF-1 enhanced differentiation of chondroprogenitor cells into cartilage within 5-6 days (9). Growth hormone and IGF-1 markedly enhance DNA synthesis, expression of cartilage-specific type II collagen, proteoglycan, chondrocalcin and 100-kD protein (10). These in vitro experiments were supported by an in vivo study, delivering growth hormone or IGF-1 into the growth plate. Growth hormone but not IGF-1 stimulated thymidine uptake to the reserve cells.

The transition in growth plate from reserve cell zone to proliferating cartilage is associated with expression of the highest concentration of IGF-1, IGF-1 receptors and the cell's responsiveness to IGF-1. Growth hormone stimulation of proliferation seems, therefore, to be an indirect effect of reserve cells that were stimulated by growth hormone to advance into their proliferative phase (10) With chondrocyte hypertrophy in focus, showed conclusively that in vivo injections of growth hormone and IGF-1 stimulate the longitudinal orientation of hypertrophic cells, thereby adding substantially to bone length.(11, 12) Similarly Javaid et al found positive correlation between cord IGF-1 levels and neonatal bone mass and body composition (13) Our findings are consistent with the idea that circulating IGF-1 appears to play a role in bone maturation and intrauterine growth.

The present study also showed that cord serum IGF-1 concentrations were all positively correlated with birth weight, body surface area and midarm circumference in term neonates. We also demonstrated a relationship between cord IGF-1 and midarm circumference but not skin fold thickness, suggesting a relationship between lean and not fat mass. This data is in accord with the results found by Javaid et al using whole body dual-energy X-ray absorptiometry. Although our findings were consistent with the idea that IGF-1 induced changes in epiphyseal area may in part mediate the anabolic effects of growth hormone on bone tissue, further studies are needed to establish the cause and effect relationship in order for IGF-1 measurements to have maximum utility.

Acknowledgments
The authors would like to thank Nick Harvey and Cyrus Cooper for their support and criticism.

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