



ORIGINAL RESEARCH

BONE TURNOVER MARKERS IN PREMATURE INFANTS

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ABSTRACT

Objective: We aimed to evaluate the bone turnover markers in preterm infants, and analyze their relationship with growth, urinary calcium (Ca) and phosphorus (P).

Subjects and Method: Thirty-nine premature infants with enteral feeding started before the 5th day of life, and 20 full-term infants, as control group, were enrolled for the study. The weight and length of all premature infants were measured at birth and repeated at 4 weeks of age. Blood samples and urine were obtained for bone turnover markers at the second measurement.

Results: Serum β -CrossLaps and urinary deoxypyridinoline, calcium, tubular phosphate reabsorption (TPR) and TPR/GFR were significantly higher in preterm infants ($P < 0.05$ for all parameters). There was significant correlation of osteocalcin with birth weight ($r = 0.306$, $P = 0.05$) and weight at 4 weeks ($r = 0.307$, $P = 0.047$); and negative correlation of urinary deoxypyridinoline with birth weight ($r = -0.310$, $P = 0.05$), weight gain ($r = -0.625$, $P = 0.001$) and weight at 4 weeks ($r = -0.387$, $P = 0.015$).

Conclusion : A significant correlation was found between urinary deoxypyridinoline and TPR ($r = 0.314$, $P = 0.05$). Preterm infants with low birth weight and low weight gain risk getting osteopenia. Measurement of TPR and calcium excretion may be a good marker of preterm osteopenia.

Keywords: Bone turnover, Growth, Prematurity, Urinary calcium

PREMATÜRE INFANTLARDA KEMİK DÖNGÜSÜNÜN BELİRTEÇLERİ

ÖZET

Amaç: Bu çalışmada prematüre infantlarda kemik döngüsü belirteçlerinin seviyesi ölçülerek büyüme, idrar kalsiyumu ve fosforu ile ilişkisine bakıldı.

Yöntem: Enteral beslenmesi yaşamın ilk 5 günü içinde başlanmış 39 prematüre infant ile 20 term infant çalışmaya alındı. Prematüre infantların doğumda ve 4. haftada boy ve kiloları ölçüldü. Kemik döngüsünün belirteçleri için 4. haftada idrar ve kan örnekleri alındı.

Bulgular: Serum β -CrossLaps, idrar deoxypyridinoline, kalsiyum, tübüler fosfat reabsorbsiyonu (TPR) ve TPR/GFR oranı prematüre infantlarda term infantlara göre istatistiksel olarak yüksekti (tüm parametreler için $p < 0.05$). Osteocalcin ile doğum kilosu ($r = 0.306$, $P = 0.05$) ve 4. haftadaki vücut ağırlığı arasında ($r = 0.307$, $P = 0.047$) pozitif korelasyon saptanırken, idrar deoxypyridinoline ile doğum kilosu ($r = -0.310$, $P = 0.05$), kilo alımı ($r = -0.625$, $P = 0.001$) ve 4. haftadaki vücut ağırlığı ($r = -0.387$, $P = 0.015$) arasında negatif korelasyon saptandı. İdrar deoxypyridinoline ile TPR'nin korele olduğu gözlemlendi ($r = 0.314$, $P = 0.05$).

Sonuç: Kilo alımı iyi olmayan prematüre infantların osteopeni yönünden risk altında olduğu düşünüldü. TPR ve idrar kalsiyum atılımının ölçümü prematüre osteopenisini saptamada kullanılabilir.

Anahtar Kelimeler: Kemik döngüsü, Büyüme, prematürite, İdrar kalsiyumu

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INTRODUCTION

The increasing survival rate of premature infants in developing countries over the past 20 years has created new medical diseases in these infants that did not previously exist. One such disease is osteopenia of prematurity, which occurs in approximately 20-30% of premature infants. Although most preterm infants with osteopenia are clinically asymptomatic, a minority of them develop radiological rickets and non-traumatic fractures¹. Radiological changes involving the long bones are not detectable unless the mineralization is reduced by at least 20%². Dual energy X-ray absorptiometry is the most sensitive method, but it is difficult to show acute changes; and reference values are not available for premature infants. Biochemical markers that reflect the skeletal metabolism and growth can be measured in the serum and urine³.

During the collagen maturation; pyridinium cross-links form between hydroxylysine or lysine residues at the C- and N- telopeptide ends of the collagen molecule. They are released during the matrix resorption and are excreted in the urine. They have been proven to be specific and sensitive bone resorption markers for the evaluation of metabolic bone diseases. Deoxypyridinoline (Dpd) is more sensitive to bone, because pyridinoline is also found in articular cartilage and soft tissues. Along with pyridinium cross-links, amino- and carboxy-terminal fragments of collagen attached with cross-links are released. Carboxy-terminal telopeptide of type 1 collagen is called β -CrossLaps. It is a useful marker for bone resorption, and can be measured in the serum³.

The aims of the study were; to compare the bone turnover markers in preterm and full-term infants and to evaluate their relationship with birth weight, gestational age and growth in order to assess the factors influencing the bone turnover in breast-fed preterm infants.

We also analyzed the relationship between the markers and serum Ca, P and 25-OH vitamin D (25-OH D) and urinary Ca, P to study whether these urinary parameters reflect the bone turnover state or not. The two markers, β -CrossLaps and urinary Dpd were used to assess the bone resorption. Osteocalcin, a noncollagenous protein of the bony matrix secreted specifically by osteoblasts, which correlates closely with osteoblastic activity, was used to assess the bone formation^{2,3}.

MATERIAL AND METHOD

Study group

We included premature neonates who were started on breast feeding before the 5th day of life. Neonates with major congenital anomalies, central nervous system disorders, and inborn errors of metabolism, suspected bone and/or muscular diseases, infants taking drugs known to affect the bone metabolism and infants of diabetic mothers were excluded from the study. Twenty healthy full-term infants were taken as the control group. All the infants were given standard vitamin D supplementation (400 IU/day for term infants and 800 IU/day for preterm infants). The gestational age was assessed by the date of the last menses and confirmed by Ballard scoring.

Study Protocol

The weight and length of all premature infants were measured by a single individual at birth. All the infants were weighed nude using standard beam balance. An infantometer was used to measure length. The measurements were repeated by the same individual at the age of 4 weeks (Table I). Blood samples were obtained for osteocalcin, β -CrossLaps, Ca, P, 25-OH D and creatinine at 4 weeks age. Urine was collected for Dpd and urinary Ca, P and creatinine over a 6 hour period with an adhesive plastic bag.



Table 1. Gestational age and anthropometric variables at birth and at the age of 4 weeks in preterm and term infants.

Parameters	Preterm infants (n=39)	Term infants (n=20)
Gestational age	31.5 ± 2.7*	38.9 ± 0.9*
Birth weight (g)	1842.8 ± 597.7	--
Weight at 4 week (g)	2052.8 ± 615.1*	3212.5 ± 559.9*
Weight gain (g)	212.5 ± 82.3	--
Birth length (cm)	40.3 ± 5.1	--
Length at 4 week (cm)	41.9 ± 4.9*	47.1 ± 1.3*
Length change (cm)	2.2 ± 1.1	--
Gender (M/F)	23/16	10/10

Results are expressed as mean ± SD

*p<0.05

Table II: Bone turnover markers, 25-OH D and urinary Ca and P in preterm and term infants at age of 4 weeks.

Parameters	Preterm infants (n=39)	Term infants (n=20)
Serum Ca (mg/dL)	9.9 ± 0.9	10.2 ± 0.4
Serum P (mg/dL)	5.1 ± 2*	6.7 ± 1.1*
Osteocalcin (ng/mL)	89.8 ± 39.5	107.2 ± 29.7
Urinary Dpd (nmol/mmol/creatinine)	197.05 ± 77.4*	113.25 ± 34.46*
β-CrossLaps (ng/mL)	0.95 ± 0.36*	0.7 ± 0.27*
25-OH D (ng/mL)	27.4 ± 15.2	27.3 ± 15.2
Calciuria (mg kg⁻¹ per day)	3 ± 1.8*	1.4 ± 1.2*
Phosphaturia (mg kg⁻¹ per day)	2.9 ± 2.7	3.5 ± 2.9
FE Ca (%)	2.9 ± 2.3	3.1 ± 2.1
TPR (%)	94.7 ± 7.9*	84.3 ± 4.3*
TPR/GFR (mg/dL⁻¹)	4.1 ± 1.4*	2.2 ± 1.0*

Results are expressed as mean ± SD

*p<0.05

Biochemical analyses

Total serum and urinary Ca was determined by colorimetric assay and P by endpoint method using Modular (Roche/Hitachi) analyzer. Urinary excretion of Ca and P was assessed on the basis of calciuria (mg/kg⁻¹ day), phosphaturia (mg/kg⁻¹ day), tubular phosphate reabsorption (TPR), fractional calcium excretion (FE Ca) and tubular phosphate reabsorption/ glomerular filtration rate (TPR/GFR). TPR was calculated (ratio of phosphate clearance to the creatinine clearance), expressed as a percentage. GFR

was calculated according to the formula defined previously⁴. Circulating osteocalcin and β-CrossLaps levels were measured by electrochemiluminescence immunoassay (ECLIA) with Modular Analytics E170, using the Elecsys N-MID Osteocalcin reagent kit. Inter-assay coefficient of variation (CV) and intra-assay CV for osteocalcin was 2.4% and 2.3%, respectively, and <20% and 17.9% for β-CrossLaps, respectively. Urinary Dpd levels were measured by HPLC, and expressed as nmol/mmol creatinine⁵. Intra-assay and inter-assay variation was 2.7-3.1% and 3.7-4.5%,



respectively. 25-OH D levels were measured by competitive binding protein assay method, using BIOMEDICA 25-(OH) D vitamin kit. Inter-assay CV was 6.6-15% and intra-assay CV was 9-14%.

All parents gave informed consent for participation in the study and the study was conducted according to the guidelines of the local ethics committee.

Statistical analysis

Data are expressed as mean \pm SD. Differences between groups were examined for statistical significance using Mann-Whitney U (for β -CrossLaps, TPR, TPR/GFR, calciuria, phosphaturia and osteocalcin) and Student's t-test (other parameters). Correlation coefficients were determined by Pearson, or Spearman's rank correlation tests were used where appropriate to determine the correlations between turnover markers, growth parameters and tubular functions. The significance threshold was retained for $P < 0.05$.

RESULTS

Thirty-nine premature and 20 full-term infants who met the inclusion criteria were enrolled into the study. The mean concentrations of serum and urinary Ca and P, and 25-OH D levels and bone turnover markers in preterm

and full-term infants are shown in Table II. The concentrations of β -CrossLaps and urinary Dpd were significantly higher in preterm infants than in term infants ($P < 0.05$) (Fig. 1). Serum osteocalcin levels were lower in preterm infants, but not statistically significant. Significant statistical differences were found between preterm and term infants in relation to calciuria (3 ± 1.8 mg/kg/day vs. 1.4 ± 1.2 mg/kg/day), TPR (94.7 ± 7.9 vs. 84.3 ± 4.3) and TPR/GFR (4.1 ± 1.4 mg/dL-1 vs. 2.2 ± 1.0 mg/dL-1) ($P < 0.05$ for all parameters).

The two markers of bone resorption, β -CrossLaps and urinary Dpd, were positively correlated with each other ($r = 0.389$, $P = 0.015$). There was a significant positive correlation of osteocalcin with birth weight ($r = 0.306$, $P = 0.05$) and with weight at 4 weeks ($r = 0.307$, $P = 0.047$) in preterm infants. On the other hand, urinary Dpd was inversely correlated with birth weight ($r = -0.310$, $P = 0.05$), weight gain ($r = -0.625$, $P = 0.001$) and with weight at 4 weeks ($r = -0.387$, $P = 0.015$) (Fig. 2).

There was no significant correlation between linear growth and bone turnover markers. A significant correlation was noted between urinary Dpd and TPR ($r = 0.314$, $P = 0.05$). No other marker showed any relationship with any measurement of urinary Ca and P.

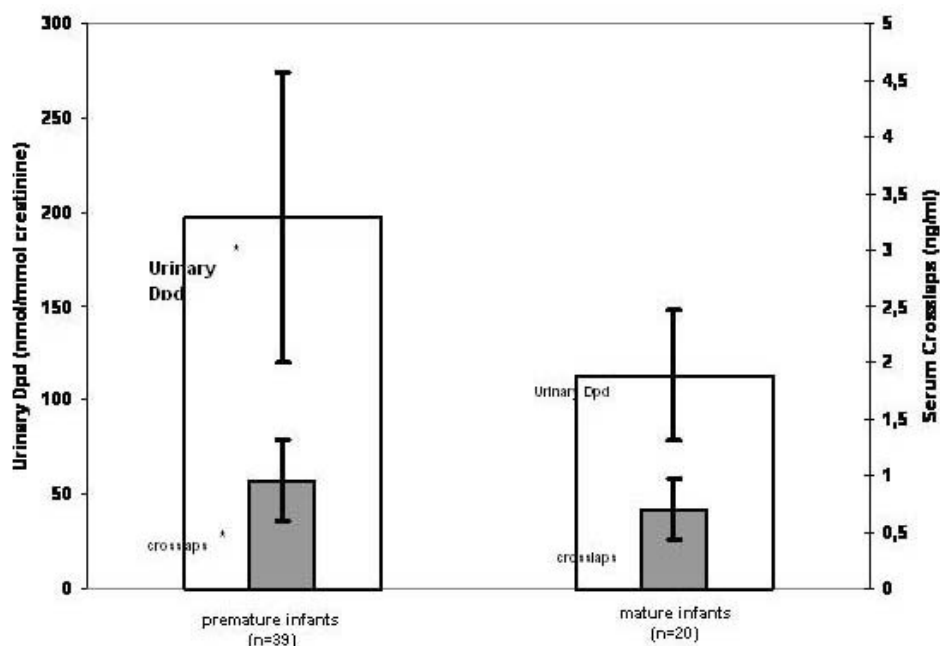


Figure 1: Bone resorption markers in preterm and full-term infants. Values are mean \pm SD. * $P < 0.05$ compared with full-term infants.

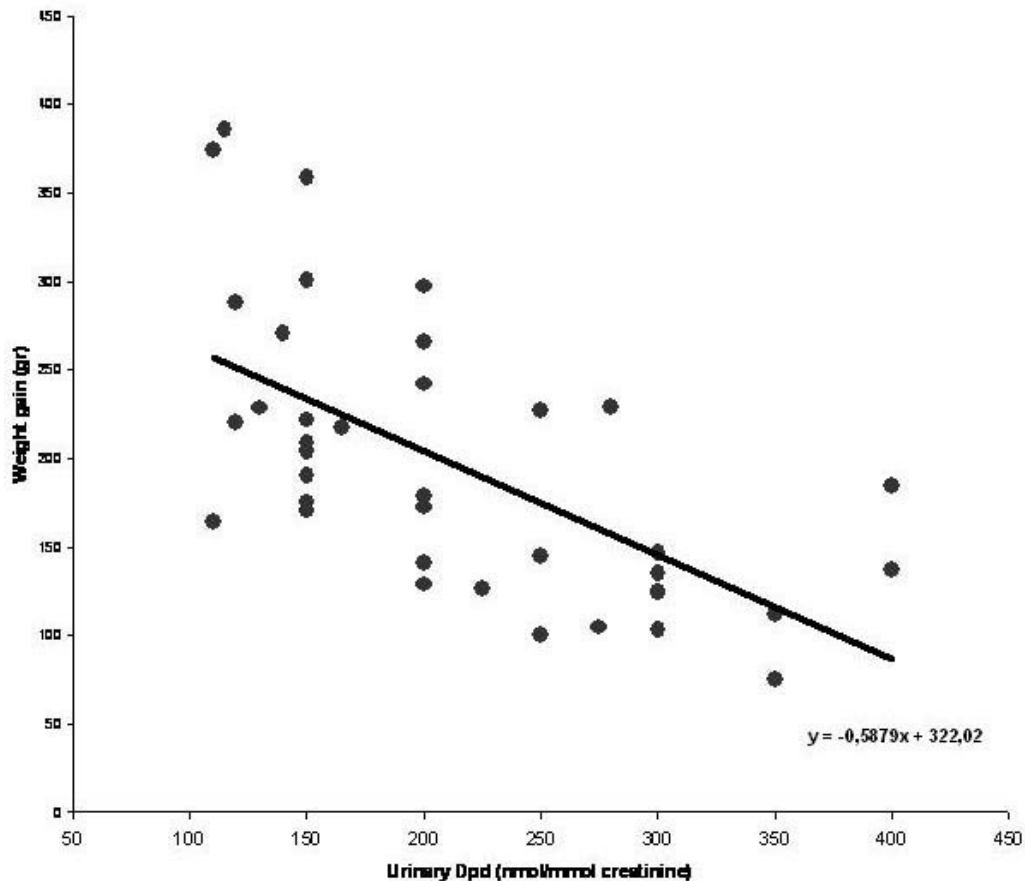


Figure 2: Relationship between urinary Dpd and weight gain in premature infants.

DISCUSSION

In the present study, we found that premature infants have decreased bone formation and increased bone resorption compared to full-term infants. This disassociation demonstrates that a state of high bone turnover underlies the development of osteopenia in preterm infants.

Preterm infants have some physical and biochemical disadvantages in bone formation compared to full-term infants. Bone loading is the primary factor for bone formation in the intrauterine period⁶. It causes a strain on the bone. It is perceived by the effector cells, osteoblasts, which cause bone formation. There are two types of bone loading in the last trimester. The first is the impact of the fetus, especially the extremities against the uterine wall. The full-term infant with an intact neuromuscular system achieves the full influence of this fetal impact on bone

formation, but preterm infants are deprived of this musculoskeletal bone loading in the last trimester. The second type of bone loading is the active and passive attachment of loads of muscles to the bone. The attaching muscles exert a small, but continuing load on the bone, even when the muscle is not actively moving the bone⁶. Weight gain is elevated during the last trimester; peak velocity is reached at the 34th postmenstrual week, reaching a plateau from the 37th week of gestation. Weight gain in the last trimester increases the bone load caused by the muscles, and thus increases the bone formation⁷. The last trimester is a critical period for the fetus regarding bone mineralization. Approximately 80% of the fetal bone is formed during this period. This period is lost and replaced with a less favorable extrauterine period. It is difficult to compensate this deficiency in the extrauterine period in preterm infants. Active and passive transplacental transport of calcium and



phosphate is increased up to 150 mg/kg/d and 75 mg/kg/d, respectively during the last trimester for bone formation⁸. For a normal extrauterine bone formation rate, preterm infants need a similar amount of calcium and phosphate, but it is difficult to achieve this level of calcium and phosphate for a breast-fed preterm infant.

Previous studies showed that concentration of bone resorption markers, C-telopeptide and N-telopeptide of type 1 collagen, at 4 weeks of age were significantly higher in preterm infants compared to full term infants⁹. Beyers et al.¹⁰ noted that preterm infants at expected full-term age had significantly greater urine excretion of hydroxyproline, and they suggested that increased bone resorption underlies the development of osteopenia of prematurity. In the present study, we found that preterm infants had significantly higher urinary Dpd excretion and β -CrossLaps levels compared to full term infants.

We found that urinary Dpd negatively correlated with birth weight, weight gain and weight at 4 weeks of age. On the contrary, no significant correlations were noted between β -CrossLaps and anthropometric measurements. Crofton et al.¹¹ studied the relation between the bone and collagen markers with growth over the first 10 weeks of life and they found that collagen formation markers positively correlated with linear growth and weight gain. Urinary pyridinoline and Dpd were inversely correlated with weight gain, as in our study. Osteocalcin was correlated with both birth weight and weight at 4 weeks of age. Seibold-Weiger et al.¹² reported concentrations of carboxyterminal propeptide of type 1 procollagen, a marker of bone formation; were significantly associated with growth velocity. There was no correlation between the turnover markers and linear growth in our study. Accurate length measurement is difficult in newborns. We believe that weight gain and linear growth increases the bone loading in postnatal period as in the last trimester. Infants with deprived weight gain had decreased bone loading, thus increased bone resorption.

In preterm infants, vitamin D and its metabolite 25-OH D concentrations in the cord blood are lower than maternal levels but correlated with concentrations in the mother. After the first week of life, plasma 25-OH D remains constant in premature infants who have received supplemental vitamin D, but 1,25 OH D concentrations increase up to 30 a day. 25-OH vitamin D concentrations have generally been reported to be normal in studies of osteopenia of prematurity in infants who had received up to 400 IU/day vitamin D¹². In our study, there was no statistical difference in the 25-OH D levels between the premature infants and the control group. No statistically significant correlations were found between the turnover markers and 25-OH D levels in our study.

Phosphate deficiency is common in breast-fed infants. Renal P reabsorption increases in response to this deficiency, and hypercalciuria may be noted paradoxically due to inadequate P to form crystal apatite¹³. The presence of about 4mg/kg/day hypophosphaturia and/or calciuria is considered an early marker of phosphate deficiency and consequently of osteopenia development¹⁴. In our study, preterm infants had increased calciuria and TPR compared to full-term infants, suggesting phosphate deficiency in preterm infants. Urinary Dpd negatively correlated with TPR. These results suggest that osteopenia in breast-fed premature infants is mainly associated with increased bone resorption rather than impaired bone formation.

In summary, we observed that preterm infants had increased bone resorption compared to full-term infants. Preterm infants with low birth weight and decreased weight gain in the postnatal period are at risk for the development of osteopenia. Measurement of urinary tubular phosphate reabsorption with Ca excretion may be helpful in the detection of bone resorption in premature infants. Further prospective studies are needed to determine the relation between early detection of bone turnover markers and fracture risks and bone mineral content in infancy.



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