Effects of Maternal Vitamin D Deficiency on Osteopenia of Prematurity in Very Low Birth Weight Premature Infants

Çok Düşük Doğum Ağırlıklı Prematüre Bebeklerde Maternal D Vitamini Eksikliğinin Prematüre Osteopenisi Üzerine Etkileri

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Abstract

Aim: This study aimed to evaluate the effects of maternal vitamin D deficiency on osteopenia of prematurity in very low birth weight premature infants.

Methods: This study was conducted on 60 premature infants born before 32 weeks of gestation with a birth weight < 1,500 g. Infants whose mothers had a 25-hydroxycholecalciferol (25-OHD) level < 40 nmol/L were assigned to group 1 (n = 30), while those with a 25-OHD level ≥ 40 nmol/L were assigned to group 2 (n = 30). Serum calcium, phosphate, and alkaline phosphatase (ALP) levels were measured on the first postnatal day, and weekly during hospitalization. Tubular phosphate reabsorption (TRP), femoral radiographs, and serum urea, creatinine, calcium, phosphate, and ALP were evaluated after 40 weeks of gestation. Two values of ALP > 800 IU/L, at least 1 week apart, was considered diagnostic of osteopenia.

Results: Osteopenia of prematurity was detected in five patients (16.7%) in group 1, and two patients (6.7%) in group 2. No statistically significant differences were found between the two groups, except for lower phosphate levels in group 1 (p = 0.04).

Conclusions: Macro-mineral deficiencies could play a key role in the development of osteopenia of prematurity. Maternal vitamin D deficiencies can have a negative impact on bone metabolism and phosphate levels in infants.

Keywords: Prematurity, osteopenia of prematurity, vitamin D deficiency

Özet

Amaç: Çok düşük doğum ağırlıklı prematüre bebeklerde, maternal vitamin D eksikliğinin prematüre osteopenisi üzerindeki etkilerini değerlendirmek.

Gereç ve Yöntem: Bu çalışma, 32. gebelik haftasından önce doğan ve doğum ağırlığı < 1.500 g olan 60 prematüre bebek üzerinde gerçekleştirildi. Annelerinde 25-OH D vitamini düzeyi <40 nmol/L olan bebekler "grup 1" (n=30), ≥40 nmol/L olan bebekler ise "grup 2" (n= 30) olarak gruplandırıldı. Serum kalsiyum, fosfor ve alkalen fosfataz (ALP) seviyeleri doğum sonrası ilk gün ve hastanede yatış süresince haftalık olarak ölçüldü. Bebekler 40. gebelik haftalarını tamamladıklarında tübüler fosfor geri Emilimi, femur radyografileri, serum üre, kreatinin, kalsiyum, fosfor ve ALP düzeyleri değerlendirildi. En az bir hafta arayla alınan iki ALP değerinin > 800 IU/L olması osteopeni tanısı için anlamlı olarak kabul edildi.

Bulgular: Grup 1'deki 5 hastada (%16,7), grup 2'deki 2 hastada (%6.7) prematüre osteopenisi saptandı. Her iki grup arasında prematüre osteopenisi gelişimi açısından fark saptanmadığı ancak 40. gebelik haftasındaki serum fosfor düzeylerinin grup 1'deki bebeklerde daha düşük olduğu (p= 0,04) görüldü.

Sonuç: Prematüre osteopenisinin gelişiminde makro-mineral eksikliği önemli rol oynamaktadır. Maternal D vitamini eksikliği, prematüre bebeklerin kemik metabolizması ve serum fosfor seviyeleri üzerinde olumsuz bir etkiye sahip olabilir.

Anahtar Kelimeler: Prematürite, Prematüre osteopenisi, D vitamini eksikliği

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Introduction

A reduction in the mortality rate of very low birth weight premature infants has led to an increase in prematurity-related diseases (1). One of these diseases is osteopenia of prematurity, which manifests as decreased bone mineralization and, in severe cases, bone fractures (1, 2).

Intrauterine bone mineralization occurs mainly during the third trimester of pregnancy. Deficient bone growth and mineralization are evident in premature infants (3). Increased postnatal nutritional requirements and conditions that prevent mineralization (e.g., delayed nutrition, malnutrition, and steroid therapy) may also contribute to osteopenia of prematurity (4).

The incidence of osteopenia is negatively correlated with birth weight and gestational age, and positively correlated with postnatal morbidities, such as bronchopulmonary dysplasia, necrotizing enterocolitis, and cholestasis (3). The reported prevalence is 20–32% in infants with a birth weight < 1,500 g, and up to 50% in infants with a birth weight < 1,000 g. A higher prevalence has also been reported in breast-fed and soy formula-fed premature infants (2,5).

The prevention and treatment of bone pathologies is a fundamental component of preterm-infant care. Normal bone growth requires adequate and balanced amounts of energy, proteins, vitamins, calcium, phosphorus, and other nutrients (6). Placental transfer, breast milk, and exposure to sunlight for cutaneous biosynthesis are important sources of vitamin D.

During the first 8 weeks of life, the serum 25-hydroxycholecalciferol (25-OHD) levels of infants and their mothers are similar. Due to sociocultural conditions and dietary habits, a high prevalence of maternal vitamin D deficiency has been reported in Turkey (7, 8).

The objective of this study was to evaluate the effects of maternal vitamin D deficiency on osteopenia of prematurity.

Material and Methods

This study is a single center, prospective and case-controlled. The study was carried out on 60 premature babies with a birth weight of <1500 g and born before 32 GW, who were hospitalized in the neonatal intensive care unit of Istanbul Bakırköy Maternity and Child Diseases Training and Research Hospital over a period of 8 months.

Infants with congenital anomalies, exitus before 40 GW after birth, incomplete data, or referral to another hospital were excluded from the study. The 25-OHD levels were measured in the mothers of the included infants. Infants of mothers with 25-OHD levels < 40 nmol/L constituted the study group (Group 1), while those with 25-OHD levels $\geq$ 40 nmol/L constituted the control group (Group 2) (9). We recorded perinatal

follow-up information for mothers, birth type, gender, GW, nutritional pattern, duration of total parenteral nutrition (TPN), medication use, need for mechanical ventilation, previous diseases, and length of stay in the intensive care unit.

All patients received elemental calcium (20–60 mg/kg/day) from the first day, and vitamin D (800 IU) from the 14th day after birth. During follow-up, phosphate (1.5–2 mmol/kg/day) was replaced in patients with a serum phosphate level < 5 mg/dL (10). Calcium, phosphate, and alkaline phosphatase (ALP) levels were measured at 7–14-day intervals to assess the development of osteopenia of prematurity. Breast milk enhancers were advised for patients with enteral nutrition > 90 mL/kg/day. Breast milk-fed infants were advised to continue with breast milk enhancers, while infants who were not fed breast milk were advised to be fed with preterm formula milk after discharge.

All infants were re-examined after 40 GW. Femur radiographs and blood and urine samples were evaluated.

Blood and urine samples

We collected 2.5 mL blood from the mothers of all infants into dry tubes, and analyzed the samples for serum calcium, phosphate, ALP, and 25-OHD on the first postnatal day. Serum calcium, phosphate, and ALP levels were also analyzed in infants on the first postnatal day. Serum urea, creatinine, calcium, phosphate, ALP, and gamma-glutamyl transferase levels, and urine phosphate and creatinine levels, were analyzed after 40 GW. Tubular phosphate reabsorption (TRP) percentages were calculated, and bone mineralization was evaluated using femoral radiographs (Table 1).

Table 1. Methods and reference ranges

Laboratory Parameters	Measurement method	Reference range
Serum calcium	O-cresolphthalein complexone	7.6–10.4 mg/dL
Serum phosphate	Molybdate UV	4.5–9.6 mg/dL
ALP	DGKC assay	0–400 U/L
Urea	Urease UV method	10–50 mg/dL
Creatinine	Compensated rate-blanked Jaffe	0.3–0.7 mg/dL
GGT	Szasz stand	5–50 U/L
Urine phosphate	Photometric UV test	40–140 mg/dL
Urine Creatinine	Compensated rate-blanked Jaffe	28–259 mg/dL
25-OHD	Micro-ELISA	< 40 nmol/L (low); $\geq$ 40 nmol/L (normal)

ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; 25-OHD: 25-hydroxycholecalciferol; ELISA: enzyme-linked immunosorbent assay

Calculation of TRP

Urine and serum phosphate and creatinine levels at 40 GW were used to calculate TRP percentages with the formula below.

TRP (% TRP) = $[1 - (\text{urine phosphate} / \text{urine creatinine} \times \text{plasma creatinine} / \text{plasma phosphate})] \times 100$

Tubular phosphorus reabsorption was found to be increased by $\geq 95\%$ (11).

Assessment of femur radiographs

Femur radiographs were obtained using the Seldix 550 device (Siemens AG, Munich, Germany) at 40 GW, and the radiographs were interpreted by the same radiologist using the scale suggested by Koo et al. (12).

Two ALP levels > 800 IU/mL, at least 1 week apart, were considered diagnostic of osteopenia (13). The parents of all infants were informed about the purpose of the study, and written consent was obtained. This study was conducted in accordance with the Declaration of Helsinki, and approval was obtained from the ethics committee of Istanbul Bakırköy Maternity and Child Diseases Training and Research Hospital.

Statistical analysis

Statistical analyses were performed using NCSS 2007 and PASS 2008 statistical software (NCSS LLC., Kaysville, UT, USA). In addition to descriptive statistics (means and standard deviations), Student's t-test was used to compare two groups with normal distributions. Groups with non-normal distribution were compared using the Mann-Whitney U test. Chi-square and Fisher's exact tests were used for comparison of qualitative data. The alpha threshold was set at 0.05.

Results

In this study, 30 infants with a mean gestational age of 30.3 ± 1.23 (range: 24–32) weeks were included in group 1 and 30 infants with a mean gestational age of 29.73 ± 1.56 (range: 25–32) weeks were included in group 2. In group 1, 14 infants were males and 24 were born by cesarean delivery; in group 2, 16 infants were males and 25 were born by cesarean delivery. Groups 1 and 2 had six and nine infants with birth weights $< 1,000$ g, respectively. There were no differences in maternal characteristics between the groups (maternal age, pregnancy follow-up, presence of preeclampsia, or maternal serum calcium, phosphate, or ALP levels).

The demographic characteristics and clinical data of both groups are shown in Table 2. There were no differences between the groups in diseases encountered during the hospital stay or drugs that could contribute to osteopenia of prematurity (Table 3).

There were no differences between the groups in first-day serum calcium, phosphorus, or ALP levels (Table 4). Osteopenia of prematurity was diagnosed in five patients (16.7%) in group 1 and two patients (6.7%) in group 2. There were no significant differences in the development of osteopenia of prematurity between the two groups.

There were no differences in serum calcium, ALP, gamma-glutamyl transferase, or TRP levels between the two groups at 40 GW. However, serum phosphate levels were lower in group 1 compared to group 2 ($p = 0.04$) (Table 5). Femur radiographs revealed stage 1 osteopenia in two patients in group 1.

Table 2. Demographic characteristics and clinical data

	Group 1	Group 2	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Gestational age (weeks)	30.3 ± 1.23	29.73 ± 1.56	0.335
Birth weight (g)	1216.67 ± 247.97	1141.67 ± 275.35	0.272
Length of hospital stay (days)	35.80 ± 19.65	38.43 ± 19.13	0.601
Achievement time of full enteral feeding (days)	22.20 ± 16.58	20.23 ± 12.83	0.609
Duration of TPN infusion (days)	17.17 ± 12.87	16.46 ± 11.15	0.823
	n (%)	n (%)	p-value++
Antenatal steroid use	18 (60.0%)	21 (70.0%)	0.417
Cesarean delivery	24 (80%)	25 (83.3%)	0.739
SGA	11 (36.6%)	13 (43.3%)	0.87

+ Student t test, ++ Chi-square test, TPN: total parenteral nutrition; SGA: small for gestational age; SD: standard deviation

Table 3. Previous diseases and medication use

	Group 1 (n = 30)	Group 2 (n = 30)	p-value
	n (%)	n (%)	
Sepsis (n = 31)	16 (53.3%)	15 (50.0%)	0.796
NEC (n = 8)	5 (16.7%)	3 (10.0%)	0.706
BPD (n = 14)	4 (13.3%)	10 (33.3%)	0.067
IVH (n = 8)	4 (13.3%)	4 (13.3%)	1.000
PDA (n = 9)	4 (13.3%)	5 (16.7%)	1.000
RDS (n = 29)	11 (36.7%)	18 (60.0%)	0.071
Steroids (n = 9)	3 (10%)	6 (20%)	0.472
Diuretics (n = 13)	7 (23.3%)	6 (20.0%)	0.754
Methylxanthine (n = 35)	17 (56.7%)	18 (60.0%)	0.793

Chi-square test or Fisher's exact test was used, NEC: necrotizing enterocolitis; BPD: bronchopulmonary dysplasia; IVH: intraventricular hemorrhage; PDA: patent ductus arteriosus; RDS: respiratory distress syndrome

Table 4. First-day calcium, phosphate, and alkaline phosphatase levels

	Group 1	Group 2	p-value
	Mean $\pm$ SD	Mean $\pm$ SD	
Calcium (mg/dL)	8.35 ± 0.96	8.42 ± 1.07	0.791
Phosphate (mg/dL)	5.07 ± 1.54	4.83 ± 1.60	0.563
Alkaline phosphatase (U/L)	209.36 ± 107.51	174.20 ± 65.15	0.131

Student's t-test was used. SD: standard deviation.

Table 5. Calcium, phosphate, ALP, GGT, and TRP levels at 40 GW

40-week parameters	Group 1	Group 2	p-value
	Mean $\pm$ SD (median)	Mean $\pm$ SD (median)	
Calcium (mg/dL)	9.65 $\pm$ 0.36	9.60 $\pm$ 0.41	0.642
Phosphate (mg/dL)	5.42 $\pm$ 1.12	5.97 $\pm$ 0.88	0.040
ALP (U/L)	458.23 $\pm$ 184.25	414.73 $\pm$ 154.40	0.326
GGT (U/L)	72.07 $\pm$ 86.57 (49)	66.46 $\pm$ 81.93 (34)	0.237
TRP (%)	86.67 $\pm$ 7.15	86.40 $\pm$ 6.58	0.881

Student's t-test and the Mann-Whitney U test were used, TRP: tubular reabsorption of phosphate; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase

Discussion

Premature infants have a greater risk of low bone mineral density, and the risk is inversely related to gestational age. Previous studies have reported a variable incidence of osteopenia of prematurity. The incidence may be up to 30% in infants with a birth weight < 1,500 g, and up to 50% in infants with a birth weight < 1,000 g. However, routine use of breast milk enhancers and preterm formula milks, and early aggressive TPN, decreased the incidence to 10–20% in infants with a birth weight < 1,000 g [13]. Aldemir et al. retrospectively evaluated 3,691 premature infants and found that the prevalence of osteopenia was 3.1%. They found that the prevalence rates of osteopenia were 18.4%, 4.8%, and 1% in infants with birth weights < 1,000 g, 1,000–1,249 g, and 1,250–1,999 g, respectively (14). We diagnosed osteopenia in 3 of 15 (20%) infants with a birth weight < 1,000 g, and in 4 of 45 (8.8%) infants with a birth weight 1,000–1,500 g, similar to the previously reported prevalence rates. Regular monitoring of calcium, phosphate, and ALP levels, breast-feeding or preterm formulas, breast milk enhancers, and short-duration TPN all reduce the risk of osteopenia developing in premature infants.

Although osteopenia of prematurity has a multifactorial etiology, macromineral deficiency is the most important factor. Other factors that increase the risk of osteopenia include intrauterine growth restriction, long-term TPN, use of methylxanthine, steroids, or diuretics, sepsis, bronchopulmonary dysplasia, necrotizing enterocolitis, cholestasis, immobility, and low breast milk calcium and phosphate levels (15–17). The role of vitamin D in the etiology of osteopenia of prematurity is not completely understood. During the first 8 weeks of life, serum 25-OHD levels of infants are similar to the maternal levels. Due to the sociocultural conditions and dietary habits in Turkey, maternal vitamin D deficiency has a high prevalence (7, 8). In a study of 87 infants, Dijkstra et al. demonstrated lower cord blood 25-OHD and higher serum ALP levels in infants with vitamin D-deficient mothers compared to controls (18). In a study of 126 females of Asian origin by Brooke et al., vitamin D and placebo were administered to 59 and 67 participants during pregnancy,

respectively. Lower 25-OHD and calcium levels, and higher ALP levels, were detected in the babies of mothers in the placebo group compared to the vitamin D group (19). Based on studies that have demonstrated similar maternal and fetal 25-OHD levels, we investigated serum calcium, phosphate, and ALP levels, instead of 25-OHD levels, in infants on the first postnatal day. We did not find any significant differences between the groups.

Serum calcium, phosphate, and ALP levels are commonly used in the diagnosis of osteopenia of prematurity. Backström et al. reported that serum ALP levels > 900 U/L were associated with low bone mineral density (sensitivity: 88%; specificity: 71%) (20). Mitchell et al. demonstrated that an isolated increase in ALP level was not associated with radiological findings of rickets and suggested weekly assessment of ALP levels (> 800 IU/L). In our study, two values of ALP > 800 IU/L, at least 1 week apart, in at-risk infants were considered significant. There was no significant difference in the rate of development of osteopenia of prematurity between the two groups.

Some studies have demonstrated normal 25-OHD levels, and normal or raised calcitriol levels, in premature infants diagnosed with osteopenia (21, 22). McIntosh et al. demonstrated normal or high 25-OHD levels in eight infants with a birth weight < 1,000 g (2,000 IU/day vitamin D administered from the 7th day) and radiological findings of rickets, suggesting that vitamin D does not play a role in the development of osteopenia (23). Hillmann et al. demonstrated that in 45% of 72 very low birth weight premature infants, daily vitamin D supplementation (400 IU) over 12 weeks was associated with either no change or a slight increase in vitamin D levels. The remaining 55% of the infants had vitamin D levels < 15 ng/ml. In the same study, vitamin D levels were not found to be correlated with serum calcium, phosphate, or ALP levels during 12 weeks of vitamin supplementation. However, low levels of serum calcium and phosphate, and high levels of ALP, were found in the vitamin D-deficient group after 12 weeks (24). The infants enrolled in our study were re-evaluated at 40 GW, as osteopenia of prematurity becomes clinically apparent at 6–12 weeks (3). The mean serum phosphate level was lower in the vitamin D-deficient than control group ($p = 0.04$), but there were no differences between the two groups in serum calcium or ALP levels, TRP percentages, or radiographical findings in the femur. Maternal vitamin D deficiency may have contributed to the low serum phosphate levels of the group 1 infants.

Although serum calcium, phosphate, and ALP levels are the most commonly used parameters for diagnosing osteopenia of prematurity, they do not have high sensitivity or specificity for osteopenia (1). Studies have demonstrated that very low birth weight preterm infants have lower renal phosphate thresholds and higher urinary phosphate excretion rates than term infants,

even in the presence of low serum phosphate levels (24, 25). Harrison et al. suggested that the TRP percentage was the best indicator of the adequacy of phosphate supplementation, and TRP percentages > 95% indicated insufficient supplementation. That study recommended that TRP levels be monitored in cases with ALP > 500 IU/L and phosphate < 1.8 mmol/L (5.5 mg/dL) (11). We measured the TRP levels after 40 GW because earlier measurements may be inaccurate. No statistically significant differences were found in TRP levels between the groups despite the low serum phosphate levels in group 1. However, these findings may be unreliable due to the small sample size of our study.

The limitations of this study included a short study period and small number of patients. Furthermore, the number of infants with a birth weight < 1,000 g, who are at the greatest risk of osteopenia of prematurity, was lower than the number of infants with a birth weight of 1,000–1,500 g. The lack of a consensus definition for the diagnosis of osteopenia of prematurity, the inadequacy of biochemical parameters and radiological imaging to diagnose osteopenia, and the use of different reference ranges for 25-OH vitamin D can be counted as other limitations.

Conclusions

In conclusion, macro-mineral deficiencies may play a key role in the development of osteopenia of prematurity. Maternal vitamin D deficiency appears to have a negative impact on bone metabolism and phosphate levels in infants. Further studies with larger samples of very low birth weight infants are required to explore this subject in more depth.

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