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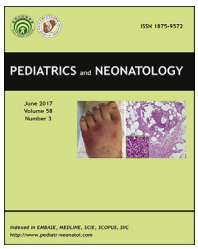

ORIGINAL ARTICLE

Bone Mineral Density According to Dual Energy X-ray Absorptiometry is Associated with Serial Serum Alkaline Phosphatase Level in Extremely Low Birth Weight Infants at Discharge

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Key Words
bone mineral density;
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premature infants

Background: To examine bone mineral density in extremely low birth weight infants at discharge and investigate whether serial measurements of serum alkaline phosphatase (ALP) and phosphate can predict bone mineralization.
Methods: The individuals were 70 preterm infants. Serum calcium, phosphate, and ALP were measured at weekly intervals during admission in extremely low birth weight infants (mean gestational age, 25.3 ± 2.1 weeks; birth weight, 812.8 ± 141.1 g). Bone mineral apparent density (BMAD) of the lumbar spine was prospectively evaluated by dual energy X-ray absorptiometry at discharge ($n = 70$).
Results: BMAD was classified as poor ($< 25^{th}$ percentile) at < 0.014 g/cm^3, fair (25^{th}–75^{th} percentile) at < 0.014–0.021 g/cm^3, and good ($> 75^{th}$ percentile) at > 0.021 g/cm^3, based on the distribution of BMAD values in infants with noncomplicated courses of prematurity ($n = 43$). In a further multivariate analysis, the number of total parenteral nutrition days, phosphate at 2 postnatal weeks and 3 postnatal weeks, and ALP at 4 postnatal weeks and 5 postnatal weeks had an impact on bone mineral density at the lumbar spine, independent of gestational age and body weight. Peak ALP activities exceeding 650 IU/L revealed low bone mineral density with 80% sensitivity and 64% specificity (AUC, 0.70; $p = 0.005$).
Conclusion: Serial measurements of serum ALP and phosphate are associated with decreased bone mineralization by dual energy X-ray absorptiometry at discharge in extremely low birth weight infants.

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1. Introduction

Osteopenia of prematurity involves a deficit of bone mineral density as a complication of premature infants born before 32 weeks of gestational age. Decreased bone mineralization is caused by calcium and phosphate accretion and inadequate supply.[1,2] After introduction of a high mineral-containing maternal diet in the late 1980s, the prevalence of osteopenia of prematurity decreased to 30% or lower.[1] Although radiologically detectable abnormalities on wrist X-ray can occur at 6—8 weeks of age in preterm neonates with overt rickets and fractures, bone mineral density must decrease by 30—50% to be detected by this method. Dual energy X-ray absorptiometry (DEXA) is the gold standard in whole-body mineral measurement, and normative data are available.[3] Although it is precise and accurate for detecting and predicting metabolic bone disease (MBD), the rationale for using a DEXA routinely in preterm infants has not been fully established due to bedside unavailability in neonatal care units.

By contrast, the threshold of alkaline phosphatase (ALP) has been used as an alternative measure to identify and guide treatment for MBD when ALP is assessed periodically. Backström et al[4] reported that peak ALP $>$ 900 IU/L was 88% sensitive and 71% specific for subsequent low bone density determined by DEXA at a 3-month corrected age. Figueras-Aloy et al[5] suggested that combined values of ALP $>$ 500 IU/L and low bone mineral density $<$ 0.068 g/cm^2 at discharge predicted the development of MBD for prematurity. There are conflicting data about serial measurements of serum ALP as a marker of early prediction of MBD in preterm infants. There are few studies on the association between serum ALP and bone mineral density with photon absorptiometry in extremely low birth weight infants. We aimed to evaluate bone mineral density in extremely low birth weight infants at discharge and determined whether serial measurements of ALP and phosphate predicted the risk of decreased bone mineral density.

2. Methods

2.1. Study population

A total of 85 infants with a birth weight $<$ 1000 g, who were admitted to the Neonatal Intensive Care Unit of Hanyang University College of Medicine, Seoul, Korea and discharged alive between 2010 and 2013, were included in this study. Of these, 70 infants completed the intervention period prospectively and underwent a DEXA scan at term. This study was approved by the Institutional Review Board for human participants at the University of Hanyang. We excluded individuals with major congenital abnormalities. For nutritional care, fortified human milk (including 82 mg calcium and 45 mg phosphorus/100 mL) or a mineral-fortified preterm formula (including 115 mg calcium and 62 mg phosphorus/100 mL) was used. Trophic feedings were initiated 1—3 days after birth and increased by 10—20 mL/kg/d. Feeding volume, advancement, and fortification were determined by neonatologists based on the same protocol. During parenteral nutrition, calcium, phosphate, and multivitamins with trace minerals were added as appropriate. A supplement of 400 IU/d of vitamin D was given to all infants. The goals for daily enteral nutrition were 120 kcal/kg/d at tolerating enteral feeding. The infants were averaging over 100—120 kcal/kg/d when they reached full-feeding. According to the protocol of the neonatal intensive care unit, stimulation of physical activity was provided by specially trained physiotherapists as soon as infants were stable enough for early developmental care. The physical activity consisted of compression and flexion/extension for range-of-motion exercises against passive resistance to upper and lower extremities. The physical activity programs provided systematic holding, massage, and tactile stimulation.

2.2. BMD measurement

Bone mineralization was assessed by total body densitometry using a commercially available DEXA (Hologic QDR-4500; Hologic, Waltham, MA, USA) in infant whole-body mode (QDR software for Windows XP, version 12.7; Hologic), with an exam performed at discharge. The exam was carried out by a technician who had no knowledge of the research data. Analysis was automatic and was performed by densitometer software. The device was calibrated daily, and the coefficient of variation remained below 2%. The patients were placed in a prone position, with lower and upper limbs extended. To secure the infants in position, they were taken to the exam after being fed, and a cotton blanket was wrapped around them from the waist down. All scans were performed without sedation. Occasionally, the scanning procedure was interrupted if a movement artifact was noted, and a repeat scan was performed when the infant had been pacified. The patient was positioned at the midline of the table, with the top of the head 5 cm from the edge of the table. DEXA evaluated bone mass of total body and of the lumbar spine (L2—L4), lean mass (g), and fat mass (g). BMD (mg/cm^2) was obtained by dividing BMC (g) by the projected bone area (cm^2). In order to eliminate the substantial effect of body weight and bone size on BMD, the volumetric bone mineral apparent density (BMAD) of the lumbar spine was estimated by dividing the BMD by the square root of the projected bone area in the particular DEXA scan.[6] Reference BMAD values were obtained from 45 clinically stable preterm infants, due to the lack of appropriate reference data for normal spinal BMD values in premature infants. The "clinically stable" preterm infants

were defined as those who had received ventilator assistance for < 28 days, tolerated full enteral nutrition before the age of 4 weeks, received no regular diuretic or cortisone treatment, and did not have septic shock or necrotizing enterocolitis (NEC). Based on the distribution of BMAD values of clinically stable preterm infants, we defined the 25th percentile and 75th percentile BMD values. The study infants were classified into the poor BMD group with < 25th percentile of BMAD ($n = 26$), the fair BMD group with 25th–75th percentile of BMAD ($n = 26$), or the good BMD group with > 75th percentile of BMAD ($n = 18$).

Data on gestational age, birth weight, sex, type of delivery, Apgar score at 1 minute and 5 minutes, preeclampsia, antenatal steroid use, and histologic chorioamnionitis were retrieved for each infant. To evaluate the effect of the course of prematurity on BMAD, we evaluated respiratory distress syndrome, patent ductus arteriosus, NEC (≥ Grade 2), sepsis, bronchopulmonary dysplasia (BPD, ≥ moderate), parenteral nutrition associated cholestasis (defined as direct bilirubin > 2.0 mg/dL), retinopathy of prematurity, and intraventricular hemorrhage (≥ Grade 2). The diagnosis of BPD was based on the need for supplementary oxygen at 28 days of age. The severity of BPD was determined at 36 weeks of gestational age; infants breathing air had mild BPD, those who needed < 30% supplementary oxygen had moderate BPD, and those needing > 30% supplementary oxygen and/or continuous positive airway pressure or a ventilator had severe BPD.[7] Intraventricular hemorrhage was defined according to Volpe.[8] NEC was defined according to Bell et al.[9] Chorioamnionitis was defined by histologic chorioamnionitis or umbilical cord vasculitis of Grade 2 or greater, according to the grading system suggested by Salafia et al.[10] We analyzed associations between supplementary oxygen days, ventilator support, hospital days, total parenteral nutrition (TPN) days, type of infant formula, and therapy with diuretics and postnatal steroids. All infants had serum total ALP, calcium, and phosphate levels reported at weekly intervals during admission. The lowest serum calcium, lowest serum phosphate, and peak ALP concentrations were recorded.

2.3. Statistical analysis

The sample size for this study was estimated based on the earlier study,[5] which described the outcome of ALP in very good BMD category (> 75th percentile), with a power of 80% ($\alpha = 0.05$, $\delta = 25$ IU/L, pooled standard deviation (SD) = 77 IU/L, $n = 25$ in infants per group). Statistics were calculated with the Student t test and Chi-square test for means and frequencies. Numeric data are presented as mean and standard deviation because the data were normally distributed. Calculations were performed with SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). All p values < 0.05 were considered statistically significant. In order to determine the risk of decreased bone mineral density, we performed multivariate linear regression analysis and entered gestational age at birth, body weight at the time of measurement, presence of BPD, TPN days, peak and trough serum concentration of phosphate at 2 and 3 weeks of postnatal age, and peak and trough serum concentration of total ALP at 4 and 5 weeks of postnatal age into the model. Receiver operator characteristic (ROC) curves were determined to illustrate the cutoff value of serum total peak ALP for detecting MBD of prematurity.

3. Results

The study population was comprised of 70 premature newborns (44 boys and 26 girls) with a mean gestational age of 25.9 ± 2.1 weeks. Thirteen (17.6%) newborns had a birth weight < 10th percentile. None presented with congenital malformations or metabolic disorders. Regarding neonatal prematurity-related diseases, 31 (44.3%) patients presented with BPD (≥ moderate), 12 (17.1%) patients with NEC, 17 (24.3%) patients with parenteral nutrition associated cholestasis, and 15 (21.4%) patients with intraventricular hemorrhage (Table 1).

The study infants were classified into the poor BMD group (< 25th percentile) with < 0.014 g/cm^3 of BMAD, the fair BMD group (25th–75th percentile) with 0.014–0.021 g/cm^3 of BMAD, or the good BMD group (> 75th percentile) with > 0.021 g/cm^3 of BMAD. This classification was based on the distribution of BMAD values of infants with noncomplicated courses of prematurity ($n = 43$). Table 2 shows DEXA data in the three study groups. The BMD and BMAD at the lumbar spine (L2–L4) in the poor BMD group were statistically and significantly lower than those in the fair and good BMD groups ($p < 0.001$ and $p < 0.001$, respectively). However, measurements of weight, height, postmentrual age (PMA), and

Table 1 Demographic, perinatal, and neonatal characteristics of the study infants ($n = 70$).

Characteristic	Value
Gestational age (wk)	25.98 ± 2.11
Birth weight (g)	812.8 ± 141.18
SGA	13 (17.6)
Birth length (cm)	32.99 ± 2.58
Birth head circumference (cm)	23.95 ± 2.63
Male gender	44 (62.9)
Cesarean section	43 (61.4)
Apgar score at 1 min	1.62 ± 0.95
Apgar score at 5 min	3.55 ± 1.37
Maternal age (y)	34.04 ± 21.53
Oligohydramnios	4 (5.7)
Preeclampsia	9 (12.9)
Antenatal steroid	48 (68.6)
Histological chorioamnionitis	43 (61.4)
RDS	64 (92.8)
PDA	53 (75.7)
NEC (≥ Grade 2)	12 (17.1)
Culture-proven sepsis	14 (20.0)
BPD (≥ moderate)	31 (44.3)
PNAC	17 (24.3)
ROP	23 (32.9)
IVH (≥ Grade 2)	15 (21.4)

Data are presented as mean ± standard deviation or n (%).
BPD = bronchopulmonary dysplasia; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; PDA = patent ductus arteriosus; PNAC = parenteral nutrition-associated cholestasis; RDS = respiratory distress syndrome; ROP = retinopathy of prematurity; SGA = small for gestational age.

Table 2 Dual energy X-ray absorptiometry (DEXA) data in the study group.

	Poor BMAD ($n = 26$)	Fair BMAD ($n = 26$)	Good BMAD ($n = 18$)	p
BMC (mg)	30.875 ± 8.220	45.267 ± 38.124	40.599 ± 22.089	0.144
BMD (mg/cm^2)	0.105 ± 0.017	0.124 ± 0.038	0.128 ± 0.377	0.043
BMCs (mg)	3.128 ± 1.084	3.796 ± 1.604	3.625 ± 1.374	0.200
BMDs (mg/cm^2)	0.087 ± 0.012	0.108 ± 0.012	0.120 ± 0.019	< 0.001
BMADs (mg)	0.014 ± 0.001	0.019 ± 0.003	0.023 ± 0.003	< 0.001
Fat mass (%)	11.67 ± 3.87	15.08 ± 5.17	12.14 ± 6.03	0.057
Wt at densitometry	2.42 ± 0.46	2.40 ± 0.42	2.35 ± 0.45	0.885
Ht at densitometry	43.94 ± 3.11	43.94 ± 2.56	43.77 ± 2.98	0.978
Hc at densitometry	31.96 ± 1.85	31.70 ± 1.45	31.77 ± 1.92	0.853
PMA at densitometry	38.15 ± 2.58	37.80 ± 3.0	37.88 ± 2.92	0.902
PNA at densitometry	85.46 ± 24.31	84.50 ± 16.24	81.33 ± 24.77	0.819

Data are presented as mean $\pm$ standard deviation or n (%).
BMADs = volumetric bone mineral apparent density; BMC = bone mineral content; BMCs = bone mineral content at lumbar spine; BMD = bone mineral density; BMDs = bone mineral density at lumbar spine; Hc = head circumference; Ht = height; PMA = postmenstrual age; PNA = postnatal age; Wt = weight.

postnatal age (PNA) based on densitometry were similar among the groups. The deleterious factors influencing BMD value are shown in Table 3. The BMD values in study groups at discharge showed a statistically significant correlation with BPD ($p = 0.035$), TPN days ($p = 0.04$), and peak ALP ($p = 0.007$). However, there were no statistically significant correlations between BMD value and gestational age, birth weight, or birth length. No statistically significant differences were found between BMD value and type of milk or therapy with diuretics and postnatal steroids. We did not find any significant difference in mean serum phosphate ($p = 0.575$), peak serum calcium ($p = 0.339$), or mean serum ALP ($p = 0.325$) through densitometry. When calcium, phosphate, and ALP were consecutively measured from Week 2 to Week 7 in Figure 1 according to BMAD groups, serial calcium levels among BMAD groups were not statistically significant. Only Week 2 and Week 3 phosphate and Week 4 and Week 5 ALP were statistically significant among BMAD groups. Thus, Week 2 and Week 3 phosphate and Week 4 and Week 5 ALP were entered into the model of multivariate logistic regression analysis for further evaluation. The poor BMD group showed significantly lower levels of phosphate from 2 ($p = 0.005$) weeks to 3 ($p = 0.007$) weeks of postnatal age and higher levels of alkaline phosphate from 4 ($p = 0.004$) weeks to 5 ($p < 0.001$) weeks of postnatal age compared to the good BMD group. Serum ALP at 5 weeks was significantly higher in the poor BMD group (676.1 ± 187.8) than in the fair (491.9 ± 172.2, $p < 0.001$) and good BMD groups (392.5 ± 112.1, $p < 0.001$).

In a further multivariate analysis, we defined decreased bone mineral density in prematurity as follows. The cutoff level for desirable apparent bone mineral density in preterm infants was obtained from the value corresponding to 25th percentile of BMAD. This value was a BMAD of 0.014 g/cm^3 at the lumbar spine at term age. Regression analysis showed that TPN days, phosphate at 2 postnatal weeks and 3 postnatal weeks, and ALP at 4 postnatal weeks and 5 postnatal weeks had an impact on BMD at the lumbar spine, independent of gestational age and body weight (Table 4).

The diagnostic performance of peak total ALP activity in detecting low BMAD at discharge is illustrated in the ROC curves (Figure 2). Peak ALP activities exceeding 650 IU/L revealed low bone mineral density with 80% sensitivity and 64% specificity ($p = 0.005$). The area under the ROC curve was 70% for peak ALP for the diagnosis of low bone mineral density.

4. Discussion

This study demonstrated that prolonged low phosphate at 2 postnatal weeks and 3 postnatal weeks and high ALP at 4 postnatal weeks and 5 postnatal weeks were correlated with decreased bone mineral density by DEXA at discharge in extremely low birth weight infants. ALP has been suggested as an alternative measure for early screening and aggressive mineral supplementation for MBD. However, the correlation between serum ALP and bone mineral density detected by DEXA remained questionable. There are conflicting data regarding the association between serum ALP and bone mineral density with photon absorptiometry in extremely low birth weight infants.[11] Clinical symptoms of osteopenia based on the radiological findings of rickets are related to morbidity in the form of fractures.[12] Although DEXA can accurately detect and monitor MBD, it is not routinely feasible in clinically unstable preterm infants who are at risk for rickets.[13] A growing body of evidence suggests that elevated ALP is a clinically useful marker for predicting the risk of osteopenia and rickets, based on radiographs at term-equivalent age.[14] However, there are still no standard cutoff levels for MBD screening with DEXA in extremely low birth weight infants.[15]

In comparison with previous studies, the poor BMD values were determined as less than the 25th percentile (< 16 mg/cm^3) of volumetric bone mineral density to estimate appropriate reference data in our findings. Furthermore, clinically stable preterm infants were first sorted by the course of prematurity to adjust for the effect of disease in this study. In particular, this study was performed to assess low mineral density as measured by DEXA at term-equivalent age, combining neonatal serial ALP measurements in a prospective design. In our study, a peak ALP level of 650 IU/L was similar to that observed in previous studies. Hung et al[16] demonstrated that a total ALP $>$ 700 IU/L at 3 weeks of postnatal age led to 73% sensitivity and 74% specificity for the

Table 3 Risk factors influencing bone mineral density in the study group.

	Poor BMAD ($n = 26$)	Fair BMAD ($n = 26$)	Good BMAD ($n = 18$)	p
Gestational age (wk)	26.30 ± 2.01	25.74 ± 2.45	25.88 ± 1.69	0.612
Birth weight (g)	795.38 ± 160.28	828.51 ± 110.26	814.70 ± 158.59	0.699
SGA	6 (23.1)	3 (11.5)	4 (22.2)	0.507
Male gender	16 (61.5)	15(57.7)	13 (72.2)	0.609
Antenatal steroid	21 (80.8)	15 (57.7)	12 (66.7)	0.354
RDS	24 (92.3)	24 (92.3)	16 (94.1)	0.969
PDA	18 (65.2)	22 (84.6)	13 (72.2)	0.400
Ligation	12 (46.2)	14 (53.8)	7 (38.9)	0.615
NEC (≥ Grade 2)	5 (19.2)	3 (11.5)	4 (22.2)	0.612
Culture-proven sepsis	6 (23.1)	5 (19.2)	3 (16.7)	0.866
BPD ≥ moderate	16 (61.5)	11 (42.3)	4 (22.2)	0.035
PNAC	7 (26.9)	7 (26.9)	3 (16.7)	0.682
ROP (≥ Stage 2)	8 (30.8)	11 (42.3)	4 (22.2)	0.363
IVH (≥ Grade 2)	8 (30.8)	3 (11.5)	4 (22.2)	0.239
Furosemide	10 (38.5)	10 (38.5)	3 (16.7)	0.237
Oxygen days	76.96 ± 28.74	68.07 ± 26.40	66.61 ± 41.59	0.481
Ventilator days	28.34 ± 20.41	23.03 ± 16.45	22.38 ± 15.53	0.451
Hospital days	94.64 ± 26.92	92.57 ± 16.57	99.55 ± 36.17	0.689
TPN days	41.61 ± 18.03	36.30 ± 12.24	29.77 ± 13.21	0.040
Diuretic use (d)	10 (38.5)	10 (38.5)	3 (16.7)	0.237
Breast milk with/without formula	25 (96.2)	24 (92.3)	16 (88.9)	0.649
Exclusively fortified breast milk	5 (19.2)	8 (30.8)	5 (27.8)	0.410
Dexamethasone use	10 (38.5)	11 (42.3)	8 (44.8)	0.918
Lowest Ca (mg/dL)	6.97 ± 1.05	6.83 ± 1.08	7.33 ± 0.98	0.294
Lowest P (mg/dL)	2.25 ± 0.65	2.71 ± 1.20	2.73 ± 0.72	0.118
Peak ALP (IL/L)	747.42 ± 200.11	674.38 ± 237.24	539.83 ± 160.39	0.007
Serum P at densitometry	9.74 ± 0.43	9.59 ± 0.54	9.48 ± 0.78	0.575
Serum Ca at densitometry	6.23 ± 0.81	6.08 ± 0.50	5.99 ± 0.92	0.339
Serum ALP at densitometry	386.53 ± 140.07	341.30 ± 99.89	344.22 ± 106.49	0.325

Data are presented as mean ± SD or n (%).
ALP = alkaline phosphatase; BMAD = bone mineral apparent density; BPD = bronchopulmonary dysplasia; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; PDA = patent ductus arteriosus; PNAC = parenteral nutrition-associated cholestasis; RDS = respiratory distress syndrome; ROP = retinopathy of prematurity; SGA = small for gestational age; TPN = total parenteral nutrition.

prediction of osteopenia. Unlike the present study, they defined osteopenia as positive radiographic findings in preterm infants that had a gestational age ≤ 34 weeks. Figueras-Aloy et al[5] identified a cutoff point of ALP > 500 IU/L for MBD in patients with body weight (BW) < 1500 g. However, their study assumed an ALP level of 500 IU/L as the cutoff point for defining osteopenia, while our study quantified decreased mineral density based on the 25th percentile and 75th percentile BMAD distribution in preterm infants. The reference BMD values were determined in patients with a serum ALP level < 500 IU/L. Mitchell et al[14] suggested that 25% (16/63) of infants with BW < 800 g showed radiologic rickets despite nutritional support, but no single ALP value was related to the radiological findings of rickets. Birth weight was the only factor triggering suspicion of rickets, rather than biochemical markers, in patients with BW < 600 g. However, their study only assessed the development of MBD based on radiographic evaluation, while our study quantified decreased bone mineral density by DEXA in extremely low birth weight infants.

Our results contradict a previously published finding in which there was no significant difference in bone mineral content between a mean ALP cutoff of < 600 IU/L and > 1200 IU/L. However, that study[17] did not measure bone mineral density, which takes bone volume into account, and did not exclude infants who had clinically complicated disease that might influence ALP with fluctuation and bone mineralization. BMD is calculated by dividing bone mineral content by the projected bone image (area in cm^2) and is dependent on bone size, which might cause erroneous interpretations of BMD values. In addition, a wide range of ALP levels might not be linearly correlated with bone mineral content. Our study revealed that prolonged elevation of ALP at 4 weeks and 5 weeks after birth was a beneficial parameter in early detection of decreased BMD. These results may be attributable to the volumetric measurement of BMAD at the lumbar spine, which reflects BMD adjusted for differences in bone size and body weight. These findings are consistent with those of Backström et al,[4] who evaluated the distribution of volumetric BMD according to DEXA. They suggested that decreased bone mineral density was correlated with a total ALP > 900 IU/L at a 3 month corrected age, after categorizing volumetric BMAD levels as above or below 95 g/cm^3. BMAD might be assessed for complex three-dimensional structures in the trabecular bone, regardless of prematurity and sex-based differences.

ALP is of limited absolute value as a stand-alone predictor of MBD, given its significant fluctuations.[18]

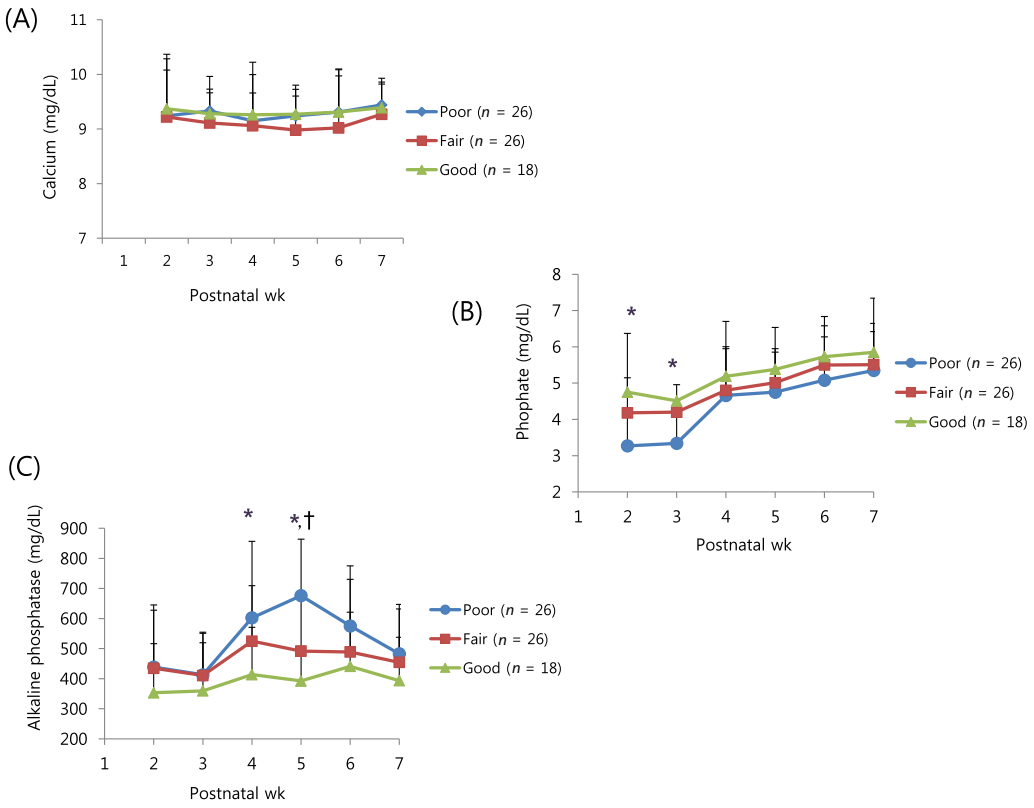

Figure 1 Comparison of serial measurement of calcium (A), phosphate (B) and total alkaline phosphatase (C) from 2 weeks to 7 weeks' postnatal age in the poor BMD (<25th percentile), fair BMD (25th–75th percentile), and good BMD (>75th percentile) groups. The poor BMD group showed significantly lower levels of phosphate from 2 to 3 weeks' postnatal age and higher levels of alkaline phosphate from 4 to 5 weeks' postnatal age (all $p < 0.01$).

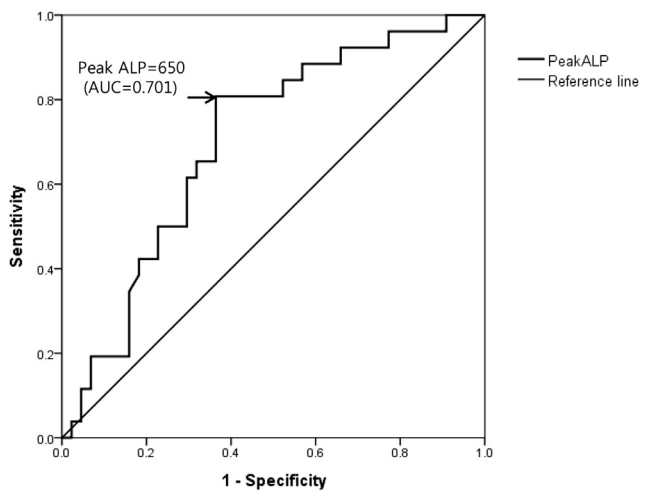

Figure 2 Receiver operator characteristic curves of peak total alkaline phosphatase for detecting the 25% of infants with the lowest bone mineral density.

Furthermore, serum phosphate concentrations should be regularly determined and maintained above 6 mg/dL (2 mmol/L).[19] A serum ALP increase at 4 weeks and 5 weeks of postnatal age was followed by prolonged reduction in serum phosphate level at 2 weeks and 3 weeks of postnatal age in the decreased BMD group, when bone mineral density was classified by values corresponding to the 25th percentile and 75th percentile. The elevation of ALP at 4 weeks and 5 weeks of postnatal age was followed by low plasma phosphate levels. Although both high ALP levels and low phosphate levels are associated with neonatal osteopenia, consecutive changes of high ALP levels followed by low phosphate levels may be considered as a marker for bony turnover for ongoing bone mineralization. Even if serum phosphate may be normal, ALP is a more reliable marker as a phosphotransferase in reflecting bone stores than serum phosphate level, which varies in renal handling of

Table 4 Multivariate logistic regression analysis of risk factors influencing bone mineral density in the study group.

	Adjusted *p*	Adjusted odds ratio (CI 95%)
Gestational age	0.125	1.294 (0.931–1.798)
Weight at densitometry	0.910	1.000 (0.999–1.002)
BPD ≥ moderate	0.118	2.735 (0.774–9.658)
TPN days	0.037	1.052 (1.003–1.104)
Phosphate at 2 wk & 3 wk	0.033	0.901 (0.819–0.992)
ALP at 4 wk & 5 wk	0.013	1.000 (1.000–1.1002)

ALP = alkaline phosphatase; BPD = bronchopulmonary dysplasia; CI = confidence interval; TPN = total parenteral nutrition.

phosphate. Backström et al[4] showed that a combination of serum ALP > 900 U/L and serum inorganic phosphate < 1.8 mmol/L at a corrected age of 3 months was 100% sensitive and 71% specific for reduced BMC, as measured by DEXA. While Hung et al[16] did not find that measurement of phosphate increased their prognostic accuracy, even 3-week-old infants in their osteopenic group had significantly lower serum phosphate than infants who did not develop MBD. Their infants also received standard calcium and phosphorus supplementation.

This study determined BMD at discharge in extremely low birth weight infants and identified factors and neonatal morbidities that affect BMD. The possible mechanism of decreased bone mineralization in infants with BPD not only includes an inadequate mineral intake along with fluid restriction, but also the use of loop diuretics or systemic steroids. Furthermore, parenteral nutrition is more prone to aggravate osteopenia if calcium and phosphorus are not adequately supplemented due to limited solubility of calcium and phosphorus. Regression analysis showed that a larger number of TPN days was a potential risk factor of poor BMD at the lumbar spine, independent of gestational age and body weight. Furthermore, provision of inadequate phosphorous that is preceded by enteral intolerance and fluid restriction in sick infants was attributable to reduced BMD. Skeletal demineralization of prematurity is associated with long parenteral nutrition, respiratory disease, NEC, infection, and steroid use, as well as a mineral deficit.[20–23] Furthermore, a peak value > 1200 IU/L in the neonatal period was associated with reduced linear growth at 18 months of age in 857 infants with birth weight < 1850 g.[18] One weakness of the present study was that the association between vitamin D levels and bone mineralization might have been neglected. Longitudinal extension of this study and prospective multicenter studies are needed to explore these complex interactions and to evaluate MBD and linear restriction in later childhood.

Our study indicates that serial measurements of ALP and phosphate may predict the risk of decreased bone mineral density by DEXA at discharge in extremely low birth weight infants. The results of this study provide reference values for volumetric BMD determined by DEXA in clinically stable preterm infants. The value of volumetric DEXA and its correlation with ALP level for predicting the presence of MBD or growth restriction in later childhood should be analyzed in a large cohort.

Conflicts of interest

No authors have a conflict of interest to declare.

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