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Original Article

A study of longitudinal measurements in the screening for osteopenia of prematurity

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Abstract

Aim: To investigate the biochemical parameters and bone "speed of sound" (SoS) values used in the screening for osteopenia of prematurity (OP).

Material and Methods: Serial measurements of tibial bone SoS and concomitant serum calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) levels of preterm infants in postnatal week 1, 3, and 6 were studied retrospectively.

Results: Fifty premature infants ≤ 35 weeks of gestational age were included in the study. A positive correlation was detected between serum P levels and SoS values at 1st and 6th week (r = 0.55, P = 0.0001 and Rho = 0.67, P = 0.0001, respectively). SoS values in postnatal week 3 were correlated with SoS values in week 1 and week 6 (Rho = 0.67, P = 0.0001 and Rho = 0.65, P = 0.0001, respectively). Serum Ca, P, and ALP levels measured in postnatal week 1 and 3 were not predictive of the subsequent risk of osteopenia. The SoS value in week 3 (≤ 2940 m/s) was predictive of the risk of osteopenia in week 6, with sensitivity of 85% and specify of 73% (Area under the curve [AUC] = 0.80, p = 0.001).

Conclusions: Serum P levels were correlated with bone SoS values. Bone SoS measurements in postnatal week 3 rather than biochemical parameters was predictive of the risk of osteopenia in postnatal week 6. For this reason, screening for osteopenia can be started at postnatal week 3 in newborn units where quantitative ultrasonography (QUS) is used.

Keywords: Osteopenia; Prematurity; Speed Of Sound; Calcium; Phosphorus; Alkaline Phosphatase.

INTRODUCTION

Premature infants have a risk of osteopenia, as their bone mineral content is lower than that of full-term infants (1). Osteopenia that can result in fractures, growth retardation, and respiratory problems can have an asymptomatic course up to 6–12 weeks until these clinical signs manifest. Therefore, infants with osteopenia risk should be screened at an earlier stage to identify OP and develop therapeutic strategies (1,2).

Although the measurement of bone mineral content by dual photon X-ray absorptiometry (DEXA) is the gold standard diagnostic method for OP, artifacts resulting from measurement, exposure to radiation, and cost restrictions hamper its use in newborns (3). Thus, serum calcium (Ca), phosphorus (P), and alkaline phosphatase (ALP) levels are the most frequently used biochemical markers in screening for OP. However, various factors give rise to difficulties in assessing these markers (1,3). Another method used in screening for OP is QUS, which can be used at the bedside and is cheaper and noninvasive. This method enables bone density and structure to be assessed quantitatively based on broadband ultrasound attenuation or SoS quantification (3,4).

Bone SoS values are low in osteopenic patients due to a reduction in bone density. SoS values are lower in preterm infants throughout the early postnatal period than in their full-term counterparts, decrease until the postnatal second month and reach the level found in term infants in the 12th postnatal month (5,6).

Only a few longitudinal studies have simultaneously measured bone SoS values and those of biochemical markers, and these studies have reported different results (7-9). It is important to understand whether serum Ca, P, and ALP levels, which are widely used in screening for OP, are predictive of both simultaneous and subsequent decreases in bone density.

This study aimed to investigate the serum Ca, P, ALP levels and bone Sos values in the screening of OP and determine whether this biochemical parameters and SoS values was reflected in variations in bone densitiy over time.

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MATERIAL and METHODS

The infants who were admitted to the neonatal unit of Erciyes University Faculty of Medicine between 2012 and 2015 and who were measured tibial SoS values and serum Ca, P, and ALP levels during first postnatal 6 weeks were reviewed for this retrospective study. Of these infants, only those with at least two serial measurements of tibial bone SoS and simultaneous serum Ca, P, and ALP measurements in the first 6 postnatal weeks were included in the study. The records of the patients were reviewed. Demographic features of patients and their mothers, serial tibial SoS values, SoS Z scores and simultaneous measurements of serum Ca, P, ALP levels of patients were recorded in study forms. Infants with any of the following were excluded: severe congenital anomalies, metabolic diseases, perinatal asphyxia, and hypothyroid diseases.

Tibial SoS measurements were performed using a quantitative ultrasound sonometry device (Sunlight Omnisence 2000, Sunlight Medical, Tel Aviv, Israel). The measurements were obtained from the right tibia. In this study, SoS values are reported as m/sec. The Z score was automatically calculated from the difference between the mean SoS value of the study population and that of an age- and gender-matched population by the device and the data were expressed as a standard deviation.

An automatic biochemical analyzer (Cobas 8000 c701, Roche, Mannheim, Germany) was used to determine the values of P (phophomolybdate), Ca (o-cresolphthaleine), and ALP (kinetic p-nitrophenilphosphate).

Infants with Z scores < -1 were considered to have a risk of osteopenia (10,11).

Statistical analysis

The Shapiro–Wilk test was conducted to determine whether the data had a normal distribution. The two-sample t-test was conducted for intergroup comparisons of parametric data. The Mann–Whitney U test was conducted for intergroup comparisons of nonparametric data. The one-way repeated measures analysis of variance test or Friedman Repeated Measures Analysis of Variance on Ranks test were conducted for analysis of serial measurements. The ROC analysis was performed to detect the diagnostic value of the measurements during the first postnatal 3 weeks in predicting the risk of OP in postnatal week 6. Using the Youden index, an optimal cut-off value for SoS was calculated. The correlation analysis of the parametric data was tested with Pearson's correlation analysis, and its correlation coefficient was expressed as "r" value. The correlation analysis of the nonparametric data was tested using Spearman's correlation analysis, and its correlation coefficient was expressed as 'Rho' value. p values < 0.05 were considered statistically significant for all tests.

RESULTS

Fifty infants of gestational age 24–35 weeks were included in the study. The demographics of the infants are presented in Table 1.

Table 1. Demographic features

Birth weight (gram)	
< 1500 grams (n = 32)	1058 ± 224
≥ 1500 grams (n = 18)	1719 ± 175
Gestational age (weeks)	
≤ 30 weeks (n = 26)	28 (26.7-30.0)
> 30 weeks (n = 24)	33 (32.0-34.7)
Male (n, %)	29 (56)
Cesarean section (n,%)	42 (84)
Maternal hypertension (n, %)	7 (14)
PPROM (n, %)	17 (34)

PPROM preterm premature rupture of membranes, SGA small for gestational age

Data on tibial SoS values in postnatal week 1 and week 3 and simultaneous serum Ca, P, and ALP levels of 50 infants were obtained from the records. Data on tibial SoS values in postnatal 9th week were available only for 29 of the 50 infants.

The times of the measurements of bone SoS values and concomitant serum Ca, P, and ALP levels were 8.2 (6.7–9.0) days in postnatal week 1, 21.0 (21.0-24.0) days in postnatal week 3, and 42.0 (42.0-44.0) days in postnatal week 6.

Bone SoS values, Z scores, serum Ca, P, and ALP levels, of infants with a gestational age > 30 weeks and gestational age ≤ 30 weeks were compared. The SoS values in the 1st week and 6th week were lower in infants of gestational age ≤ 30 weeks (p = 0.002 and p = 0.01, respectively) (Table 2).

Differences between repeated measurements of SoS, Z score values, serum Ca, P, and ALP levels were revealed. There was a significant difference between at least two measurements in three repeated measurements of each parameter (Table 3).

There was no gender difference in serial SoS values. Birth weight was not correlated with any of the serial SoS values. However, gestational week was positively correlated with SoS values in the week 1, 3, and 6, (r = 0.55, p = 0.0001; Rho = 0.44, p= 0.001; and r = 0.58, p = 0.001; respectively).

The correlation of infants' serial SoS values and concomitant serum Ca, P, and ALP levels were evaluated. There was a positive correlation of SoS values with serum P levels only in the 1st week (r = 0.55, p = 0.0001). SoS values in the 3rd week were correlated with SoS values in the 1st week (Rho = 0.67, p = 0.0001). SoS values in the 6th week were positively correlated with both serum P levels (r = 0.36, P = 0.05) and SoS values in the 3rd week (Rho = 0.55, P = 0.0001). None of the biochemical markers in the week 1 and 3 were correlated with SoS values in subsequent weeks.

There was no difference between Ca, P, and ALP levels of infants whose Z scores in the weeks 1, 3, and 6 were < -1 and ≥ -1 (Table 4).

It was evaluated that whether the infants with osteopenia in the week 6 were predicted by serum Ca, P, ALP levels and SoS values in the weeks 1 and 3. None of the biochemical parameters in the weeks 1 and 3 were predictive the infants with OP in the week 6. However, only an SoS value of ≤ 2940 m/s in the week 3 was predictive infants with osteopenia in the 6th week (AUC = 0.80, p = 0.001), with sensitivity of 89% and specificity of 73% (Table 5, Fig.1).

Table 2. Serum Ca (mg/dl), P, ALP levels and tibial SoS values of all of the infants

	≤30 weeks	n	> 30 weeks	n	P
1st week	9.54±0.67	26	9.52±0.74	24	0.89*
3rd week	9.66±0.70	26	9.62±0.58	24	0.98*
6th week	9.20±0.65	18	9.26±1.13	11	0.28*
P (mg/dl)					
1st week	4.50±0.81	26	4.30±1.42	24	0.55+
3rd week	4.97±1.24	26	5.45±1.09	24	0.15+
6th week	5.40±1.37	18	5.71±1.44	11	0.44*
ALP (U/L)					
1st week	338.69 ±132.15	26	258.16±97.47	24	0.04*
3rd week	405.19±159.21	26	376.87±148.79	24	0.52+
6th week	395.11±165.18	18	395.82±132.72	11	0.64*
SoS (m/s)					
1st week	2899.38±128.38	26	3026.79±153.38	24	0.002+
3rd week	2905.04±139.32	26	2981.83±156.89	24	0.07+
6th week	2865.78±106.36	18	2976.55±120.56	11	0.01+

Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; SoS, speed of sound
*Mann-Whitney U test, +Independent samples t test

Table 3. Serum Ca, P, ALP levels and tibial SOS values of the infants whose serial 3 times SoS measurement

	1st week (n=29)	3rd week (n=29)	6th week (n=29)	P
Ca (mg/dL)	9.49±0.64[ab]	9.67±0.73[a]	9.22±0,84[b]	0.01+
P (mg/dL)	4.52±1.06[a]	5.17±1.23[b]	5.52±1,38b[c]	0.01*
ALP (U/L)	300.04±122.56[a]	397.79±146.83[b]	395.38±151.18[b]	0.004+
SoS(m/s)	2957.03±144.05[a]	2941.90±151.52[ab]	2907.79±122.68[b]	0.04+
Z score	0.13±1.10[a]	-0.45±1.17[b]	-1.01±1.03[c]	0.001*

Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; SoS, speed of sound
*One way Repeated Measures Analysis of Variance test
+Friedman Repeated Measures Analysis of Variance on Ranks test

Table 4. Serum Ca, P, and ALP levels of the infants with and without risk of osteopenia

	1st Week (n = 50)			3th Week (n = 50)			6th Week (n = 29)		
	ZS < -1 (n = 9)	ZS ≥ -1 (n=41)	P	ZS < -1 (n=15)	ZS ≥ -1 (n=35)	P	ZS < -1 (n=18)	ZS ≥ -1 (n=11)	P
Ca (mg/dL)	9.34±0.44	9.57 ±0.74	0.38*	9.81±0.53	9.57 ±0.68	0.10+	9.16±1.03	9.33±0.42	0.67*
P (mg/dL)	4.80±1.24	4.32 ±1.11	0.25*	5.50 ±0.98	5.05 ±1.24	0.22*	5.69±1.34	5.23±1.46	0.40+
ALP(U/L)	347 ±152	289±114	0.41+	389 ±141	392 ±151	0.85+	382±150	416±157	0.64*
SoS (m/s)	2768 ±47	3002±135	0.0001*	2799 ±47	3003 ±139	0.0001*	2837±68	3022±103	0.0001+

ZS, Z score; Ca, calcium; P, phosphorus; ALP, alkaline phosphatase; SoS, speed of sound.
The infants whose Z scores < -1 were evaluated as having " risk of osteopenia"
*Mann-Whitney U test, +İndependent two samples test.

Table 5. SoS values at the week 1 and 3 in prediction for risk of osteopenia at postnatal week 6

	Cut-off	AUC	95% CI	P	sensitivity	95% CI	spesifity	95% CI	+PV	-PV
1st week SoS (m/s)	≤2998	0.70	0.50-0.86	0.05	83.3	59-96	54.5	23-83	75.0	66.7
3rd week SoS (m/s)	≤2940	0.80	0.61–0.92	0.001	89%	65–98	73%	39-94	84.2	80.0

SoS, speed of sound; AUC, area under the ROC curve; CI, confidence intervale

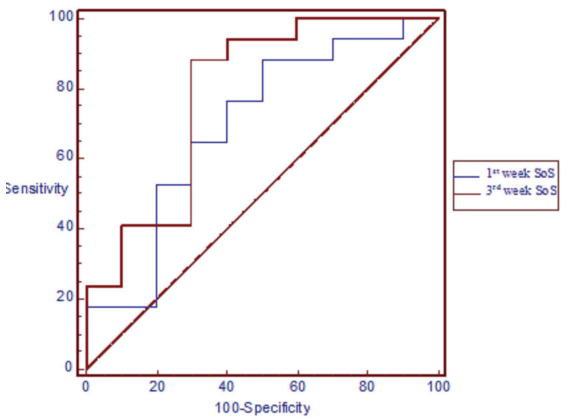

Figure 1. Roc curves of SoS values at the week 1 and 3 in prediction for risk of osteopenia at the week 6

DISCUSSION

There are various suggestions of approach to scanning and diagnosis of premature osteopenia. biochemical parameters are most frequently used in the screening of premature osteopenia. In general, at the time of diagnosis, the level of serum calcium (Ca) level is normal, phosphor (P) level is either normal or low, alkaline phosphatase (ALP) level is high. Serum calcium level is not a practical screening test in infants capable of maintaining a normal serum calcium level when the loss of bone calcium occurs. Assessment of low serum phosphate level together with high ALP level raises specificity and sensitivity. However, the uncertainty regarding the cut-off values still continues (1,3,4).

Elevations of serum ALP and clinical findings of osteopenia are uncommon in the first 4 weeks after birth at any gestational age. Therefore, American Academy of Pediatrics recommend that screening the serum ALP and serum phosphorus at 4 to 6 weeks after birth in premature infants followed by biweekly monitoring is appropriate (12). Another method used in screening for OP is QUS. It is not clear when QUS should begin to scan premature osteopenia."

In this study, serum Ca, P, ALP levels and tibial bone SoS values measured in the 1st, 3rd, and 6th weeks of preterm infants ≤ 35 weeks of gestational age were reviewed retrospectively. The results revealed a positive correlation between serum P levels in week 1 and week 6 and concomitant SoS values. SoS values in postnatal week 3 were positively correlated with both week 1 and week 6 SoS values. A SoS value ≤ 2940 m/s in the 3rd week was predictive infants with osteopenia in the 6th week, with sensitivity of 89% and specificity of 73%.

In the present study, tibial bone SoS values of infants of gestational age ≤ 30 weeks were relatively lower than those of infants of gestational age > 30 weeks. All serial SoS values were positively correlated with gestational ages but not with birth weights. Previous studies demonstrated the tibial bone SoS values exhibited a stronger positive correlation with gestational age than with birth weight (13-15). Approximately 80% of Ca and P in the fetal bone accumulate in the third trimester. Therefore, the most important risk factor for OP is prematurity (16).

The present study revealed no difference between serial SoS values of females and males. Although one previous study reported that males had higher SoS values at birth (15), the majority of studies found no sex-related difference in SoS values (12,14,17).

To assess longitudinal changes in serum Ca, P, and ALP levels and SoS values, we evaluated differences between repeated measurements. The results revealed a progressive increase in serum P levels and ALP levels. The progressive increase of P levels can be explained by increased enteral feeding and oral P supplementation, whereas the increase of ALP levels can be explained by increased osteoblastic activity following a reduction in bone density. Previous studies of longitudinal bone SoS values in preterm infants demonstrated that SoS values decreased progressively up to the second month (5,6), which was in agreement with the findings of the current study.

Although the most frequently used screening parameters for OP are serum Ca, P, and ALP levels, it is difficult to interpret the levels of these biochemical markers. In general, the serum Ca level is normal, P level is decreased or normal, and ALP level is elevated at the time of a diagnosis of OP (16,18,19). An assessment of serum P levels, together with serum ALP levels, increases the sensitivity and specificity of an OP diagnosis (1,16). In the current study, there was a positive correlation between serum P levels and SoS values in both week 1 and week 6. This finding was not surprising, as insufficient phosphate is a critical factor in OP. However, serum Ca and ALP levels were not correlated with SoS values. Faerk et al. (20) compared bone mineral content (BMC) measurements obtained by DEXA and serum ALP levels in preterm infants. They found no significant association between BMC measurements and the highest and lowest levels of ALP, and they concluded that ALP alone was not a helpful guide to aid decision making regarding commencement of treatment. Moreover, McDevitt et al also found no association between peak ALP levels and SoS values (8).

Several studies reported that low levels of serum P or high levels of ALP were related to a low mineral content in subsequent periods in preterm infants. Backstrom et al. (4) demonstrated that low levels of P were related to low BMC in the 3rd and 6th month (4). In another study, it was reported that hypophosphatemia in the 6th postnatal week was associated with low BMC throughout infancy (21). Hung et al also demonstrated that ALP levels over 700 IU/L in the 3rd week were predictive of the subsequent risk of osteopenia in preterm infants (22). Since it is need to detect the risk of OP as early as possible in preterm infants, these findings in the studies mentioned above are considerably. In the current study, serum Ca, P, and ALP levels in the 1st and 3rd postnatal week were not correlated with subsequent SoS values. In addition, serial serum Ca, P, and ALP levels were not predictive of the risk of osteopenia in the 6th week.

Chen et al. (15) demonstrated that gestational age and SoS values at birth were the most important factors in predicting SoS values throughout infancy (15). In the current study, there was a positive correlation between serial SoS values and serum P levels, and SoS values in the 3rd week were significantly predictive of the risk of osteopenia in the 6th week.

The limitations of the current study are its retrospective design and sample size. Especially the number of measurements in the sixth week is smaller. However, a positive aspect of the study was that both serial SoS measurements and biochemical measurements of infants were obtained at the same time.

CONCLUSION

In conclusion, among serum Ca, P, and ALP levels, only serum P levels were correlated with SoS values. Serum Ca, P, and ALP levels, which are frequently used markers in screening for osteopenia, did not predict the concomitant and subsequent risk of osteopenia. Bone SoS values measured as early as postnatal week 3 predicted the risk of osteopenia in the postnatal week 6. For this reason, we suggest that screening for osteopenia can be started at postnatal week 3 in newborn units where QUS is used.

Competing interests: The authors declare that they have no competing interest.
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REFERENCES

1. Vacchharajani AJ, Mathur AM, Rao R. Metabolic bone disease of prematurity. Neoreviews 2009;10:402-11.
2. Bowden LS, Jones CJ, Ryan SW. Bone mineralisation in ex-preterm infants aged 8 years. Eur J Pediatr 1999;158:658-61.
3. Harrison HM, Gibson AT. Osteopenia in preterm infants. Arch Dis Child Fetal Neonatal Ed 2013;98:272-5.
4. Backstrom MC, Kouri T, Kuusela AL, et al. Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. Acta Pediatr 2000;89:867-73.
5. Nemet D, Dolfin T, Wolach B, et al. Quantitative ultrasound measurement of bone speed of sound in premature infants. Eur J Pediatr 2001;160:736-40.
6. Rubinacci A, Moro GE, Boehm G, et al. Quantitati ve ultrasound for the assessment of osteopenia in preterm infants. Eur J Endocrinol 2003;149:307-15.
7. Fewtrell MS, Loh KL, Chomtho S, et al. Quantitati ve ultrasound (QUS): a useful tool for monitoring bone health in preterm infants? ActaPaediatr 2008;97:1625-30.
8. McDevitt H, Tomlinson C, White MP, et al. Changes in quantitative ultrasound in infants born at less than 32 weeks' gestation over the first 2 years of life: influence of clinical and biochemical changes. Calcif Tissue Int 2007;81:263-9.
9. Ashmeade T, Pereda L, Chen M, et al. Longitudinal measurements of bone status in preterm infants. J PediatrEndocrinolMetab 2007;20:415-24.
10. AltuncuA, Akman I, Yurdakul Z, et al. Quantitative ultrasound and biochemical parameters for the assessment of osteopenia in preterm infants. J Maternal Fetal Neonatal Med 2007;20:401-5.
11. Specker BL, Schoenau E. Quantitative bone analysis in children: current methods and recommendations. J Pediatr 2005;146:726-31.
12. Abrams SA, Committee on Nutrition, AAP. Calcium and vitamin d requirements of enterally fed preterm infants. Pediatrics 2013;131:1676-83.
13. Pereda L, Ashmeade T, Zaritt J, et al. The use of quantitative ultrasound in assessing bone status in newborn preterm infants. J Perinatol 2003;23:655-9.
14. Gursoy T, Yurdakok M, Hayran M, et al. Bone speed of sound curves of twin and singleton neonates. J Pediatr Endocrinol Metab 2008;21:1065-71.
15. Chen HL, Tseng HI, Yang SN, et al. Bone status and associated factors measured by quantitative ultrasound in preterm and full-term newborn infants. Early Human Dev 2012;88:617-22.
16. Rigo J, Mohamed MW, Curtis M. Disorders of calcium, phosphorus and magnesium metabolism. in: Martin RJ, Fanaroff AA, Walsh MC eds. Neonatal Perinatal Medicine Disease of the Fetus and Infant. St. Louis, Missouri: Elsevier Mosby 2011;1523-56.
17. Littner Y, Mandel D, Mimouni FB, et al. Bone ultrasound velocity curves of newly born term and preterm infants. J Pediatr Endocrinol Metab 2003;16:43-7.
18. Tinnion RJ, Embleton ND. How to use... alkaline phosphatase in neonatology. Arch Dis Child Educ Pract Ed 2012;97:157-63.
19. 19- Sharp M. Bone disease of prematurity. Early Hum Dev 2007;83:653-8.
20. Faerk J, Peitersen B, Petersen S, et al. Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. Arch Dis Child Fetal Neonatal Ed 2002;87:F133-6.
21. Kurl S, Heinonen K, Lansimies E. Pre- and post-discharge feeding of very preterm infants: impact on growth and mineralization. ClinPhysiolFunct Imaging 2003;23:182–18
22. Hung YL, Chen PC, Jeng SF, et al. Serial measurements of serum alkaline phosphatase for early prediction of osteopaenia in preterm infants. J Paediatr Child Health 2011;47:134-9.