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ORIGINAL ARTICLE

Parathyroid hormone as a marker for metabolic bone disease of prematurity

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OBJECTIVE: To compare parathyroid hormone to alkaline phosphatase as a serologic marker for metabolic bone disease (MBD) in preterm infants.

STUDY DESIGN: An 18-month prospective observational study in neonates with birth weight <1250 g. Simultaneous serum parathyroid hormone (PTH), alkaline phosphatase (ALP), calcium (Ca) and phosphorus (P) were measured at scheduled intervals during hospitalization. At 6 weeks of age, MBD was evaluated using knee radiographs. Comparisons were analyzed using multivariate logistic regression, receiver operating characteristic (ROC) curves, χ^2 and Student *t*-test.

RESULT: Fourty-nine infants were included in the study: 7 with severe and 42 with mild MBD. Using ROC curves, at 660 U l^{-1} ALP had a sensitivity of 29% and specificity of 93% for severe MBD, while a cutoff point of 180 mg dl^{-1} gave PTH a sensitivity of 71% and specificity of 88%. Infants with severe bone disease had a lower birth weight, 21-day serum P, an increased use of glucocorticoids and caffeine, and more likely to have major neonatal morbidities.

CONCLUSION: PTH is an early marker with better sensitivity than ALP in screening for MBD. At 3 weeks chronologic age, a PTH level >180 mg dl^{-1} or a P level <4.6 mg dl^{-1} yielded a sensitivity of 100% and specificity of 94% for severe MBD.

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INTRODUCTION

Even with advances in neonatal nutrition, metabolic bone disease (MBD) continues to be a frequent morbidity in premature neonates.[1–3] MBD, previously known as osteopenia or rickets of prematurity is characterized by a reduction in osteoid deposition and/or a defect in bone mineralization.[4,5] MBD has a multifactorial etiology, including prematurity, inadequate nutrition, chronic illnesses and exposure to osteolytic medications. Fractures, prolonged ventilator dependence, poor extrauterine growth, and short stature are serious complications of MBD that may affect long-term bone health.[3,6–9]

In April of 2013, the American Academy of Pediatrics-Committee on Nutrition released a statement reinforcing the significance of optimizing nutrition in preterm neonates to improve bone health.[10] The rate of calcium (Ca) and phosphorus (P) deposition in bone increases during pregnancy, with 80% of Ca accrual occurring in the last trimester. Therefore, preterm infants receive an incomplete transfer of Ca and P and consequently have an increased risk for developing poorly mineralized bones. After birth, the placental supply of Ca ends and very low birth weight neonates must now rely on receiving most of their nutrition parenterally. Although this intervention allows for steady extra-uterine growth, it does not provide the quantities of Ca nor P necessary for normal bone mineralization. Enteral feeds are begun within the first few days of life and gradually advanced; however, their immature digestive system has poor absorptive properties and human milk is low in Ca and P concentration. As a result, infants undergo a hypocalcemic phase that normalizes *via* the activation of parathyroid hormone (PTH).[11–15]

The parathyroid gland is sensitive to minor fluctuations in serum ionized Ca and during periods of prolonged Ca deficiency the production of PTH is upregulated. Thereafter, PTH promotes the proliferation and differentiation of osteoclasts, the cells responsible for removal of Ca from bone.[16–18] This process results in breakdown of bone that is already under-mineralized in premature infants.

Currently, neonatologists use a combination of serologic and radiologic indices to screen and diagnose MBD. The most commonly used and studied serologic marker is alkaline phosphatase (ALP).[1,4,19,20] It is found on the membrane of osteoblasts and rises during high bone turnover rates.[20,21] Although ALP is routinely used, it may not reach its peak until 6–12 weeks and at lower levels has sub-optimal sensitivity and specificity for detecting MBD.[22–24]

Given that PTH increases bone turnover, investigation of its utility as a serologic marker for MBD is warranted. Furthermore, the use of PTH to assess MBD has not been studied in very preterm infants. The aim of this study was to compare PTH with ALP as an early serologic marker for severe MBD in preterm infants with a birth weight <1250 g.

METHODS

Study center and subjects

This study was conducted at the neonatal intensive care unit at The University of Texas Medical Branch (UTMB) at Galveston. The institutional review board at UTMB approved the study. Infants who were included in the study weighed $\leqslant 1250$ g birth weight, survived, and were born between 1st January 2011 and 30th June 2012. Neonates with major congenital anomalies, infants who were transferred into or out of UTMB's unit and subjects who did not receive a standardized lower extremity radiograph at 6 weeks were excluded.

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Study design

This was an 18-month prospective study in very preterm neonates designed to compare PTH with ALP in assessing bone demineralization. Our management protocol for MBD in our unit is that all infants in the study had simultaneous serum Ca, P, ALP and intact PTH measured every 3 weeks during their hospitalization. If a patient had an elevated PTH value, we would change the interval of lab draws from every 3 weeks to every 2 weeks, until two consecutive PTH values were within normal limits.

Serum intact PTH values were measured via chemiluminescent immunoassay using Access intact PTH on the Unicel DxI 600 (Beckman Coulter, Brea, CA, USA). The imprecision is $\leqslant 8\%$ for PTH values $>12\,mg\,dl^{-1}$. Serum ALP values were measured using VITROS alkaline phosphatase slides (Ortho Clinical Diagnostics, Raritan, NJ, USA). The within-subject variation reported by the manufacturer is between 1.4 and 2.6%. Serum Ca and P were analyzed with a Vitros 5,1 FS Chemistry System (Ortho Clinical Diagnostic). The within-subject variability in adults is 1.4–1.6% for Ca and 1.5–2.4% for P.

MBD was evaluated using lower extremity (knee) radiographs, as the knee and wrists are known areas of rapid bone growth.[25–27] Although radiological bone changes must be significant (20–50%) to detect MBD, this was the imaging model that was accessible at our institution.[28] We chose 6 weeks of age as our evaluation point to capture the earliest changes in bone mineralization.[29,30]

The radiologic technicians performed standardized radiographs with the same exposure, development and positioning of the lower extremity for review. A leading authority in pediatric radiology was blinded to patient information and classified MBD into the following categories: none, mild or severe (refer to Figure 1).

Ca intake was optimized in our patient population. Standardized management of very low birth weight infants born at UTMB's NICU have been previously described,[31] with the following additions: (1) total parenteral nutrition initiated on the second day of life includes 40 mg per total grams of amino acids of cysteine, 140 IU of vitamin D, 0.3–0.5 meq kg^{-1} of magnesium, 1–3 meq kg^{-1} of Ca and $1-2$ mMol kg^{-1} of P is added to total parenteral nutrition dependent on patient electrolytes and solubility in parenteral fluids; (2) mixing preterm formula to a concentration of 24 calories occurs once the infant starts tolerating 100 cc kg^{-1} per day (146 mg of Ca per 100 ml, 81 mg of P per 100 ml); (3) infants on human milk are supplemented with fortified donor milk (128 mg of Ca per 100 ml, 72 mg of P per 100 ml) at 100 cc kg^{-1} per day; (4) Infants with PTH levels $>88\,mg\,dl^{-1}$ (upper limit of adult normal as per manufacturer) were started on calcium carbonate ($CaCO_3$). Values $>88\,mg\,dl^{-1}$ were used owing to a lack of standardized range for neonates. $CaCO_3$ is used because it has a high concentration of elemental Ca (40%) and was initiated once infants achieved full enteral feeds (110 kcal kg^{-1} per day). The amount of elemental Ca received by infants can be reviewed in our previous publication.[32]

Data collected during the first 3 weeks included total daily intake of Ca, P, and total parenteral and enteral calories. These values required calculating the actual mineral content of any formula consumed plus the mineral content in the infant's total parenteral nutrition. To accomplish this, the consumed quantities of formula(s) (or human milk) plus any fortifiers were obtained for each infant daily for the first 3 weeks. Intact PTH, ALP and serum electrolyte values were collected for each neonate. Family demographics, maternal obstetrical history, infant history and diagnoses, and medications known to be associated with MBD (steroids, methylxanthines, diuretics) were also collected.

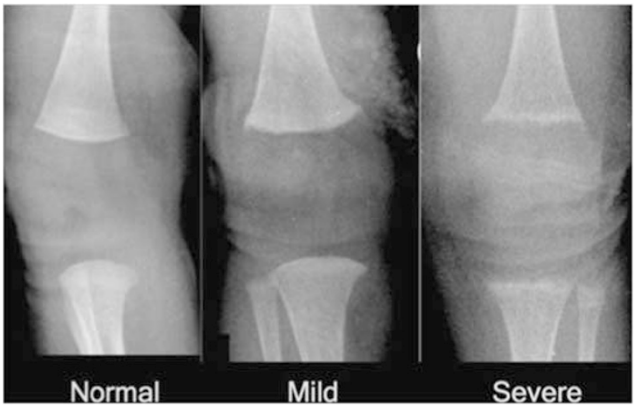

Figure 1. MBD classification. Normal—femur is dense and white with defined cortex, zone of provisional calcification is present. Mild— femur is demineralized, no zone of provisional calcification, edge of the metaphysis is irregular and indistinct. Severe—grossly demineralized bone, no zone of provisional calcification, brush border exists at edge of metaphysis.

Statistical analysis

Continuous data was analyzed using Student's *t*-test and categorical data was analyzed using χ^2 analysis and Fisher exact test for small sample cross tables. Data are reported as mean ± standard deviation. Receiver operating characteristic curves and area under the curves were generated to compare the accuracy of ALP and PTH as serologic markers for severe MBD. Multivariate logistic regression analysis was used to examine the risk of severe MBD independent of variables found to be significantly associated with MBD. Analyzes were performed using SPSS 16.5 statistical software (SPSS, Chicago, IL, USA) and an arbitrary *P*-value of < 0.05 was used to indicate significance.

RESULTS

Subjects

During this 18-month period, a total of 75 infants with a birth weight < 1250 g were cared for at UTMB. Forty-nine neonates (65%) met the inclusion criteria and they all had MBD on radiograph. Seven (14%) infants were classified with severe MBD on knee radiograph. Infants with severe MBD had a lower birth weight and were growth restricted *in-utero* ($P < 0.05$). The demographic data is summarized in Table 1.

PTH and ALP levels

Values for all PTH labs drawn ranged from 4–442 $mg\,dl^{-1}$, with a mean of $130 \pm 92\,mg\,dl^{-1}$. Infants with severe MBD had a higher PTH value when compared with the mild MBD group. Values for all ALP levels drawn ranged from 44–2570 U l^{-1}, with a mean of 423 ± 173 U l^{-1}. There was no statistical difference in ALP levels between the two groups. Refer to Table 2.

Receiver operating characteristic curves

The use of serum ALP and PTH at 3 weeks of age in predicting severe MBD at 6 weeks chronologic age is shown in the receiver operating characteristic curve in Figure 2. The sensitivity and specificity of ALP and PTH as tests for severe bone demineralization were maximized at values of 660 U l^{-1} and 180 $mg\,dl^{-1}$, respectively. At these levels, PTH was more sensitive and specific than ALP (Table 3). Based on the receiver operating characteristic curve, inorganic P as a sole marker for severe MBD correlated poorly. However, a

Table 1. Patient characteristics

Metabolic bone disease	*Mild*	*Severe*
Number of patients, No. (%)	42 (86)	7 (14)
Gestational age, mean (s.d.), weeks	26.6 (1.6)	25.4 (1.4)
Birth weight, mean (s.d.), g	872 (196)	704 (175)[a]
Male gender, No. (%)	22 (52)	4 (57)
Cesarean delivery, No. (%)	31 (74)	6 (86)
Race/ethnicity, No. (%)		
Caucasian	10 (24)	1 (14)
African American	13 (31)	1 (14)
Hispanic	17 (40)	4 (57)
Apgar score at 5 min, mean (s.d.)	7.4 (1.3)	7.0 (1.4)
Antenatal steroid, No. (%)	41 (98)	5 (71)[a]
Pregnancy-induced hypertension, No. (%)	16 (38)	2 (29)
Intrauterine growth restriction, No. (%)	1 (2)	2 (29)[a]

[a]$P < 0.05$

PTH level >180 mg dl^{-1} or a P level <4.6 mg dl^{-1} produced a sensitivity of 100% and specificity of 94%.

Nutrition data

Days to reach full feeds and regain birth weight were comparable between the two cohorts. Additionally, there was no difference in average daily enteral, parenteral, Ca nor P intake over the first 3 weeks of life (Table 4).

Table 2. Biochemical indices at 21 days

Metabolic bone disease	*Mild,* n = *42*	*Severe,* n = *7*	P-*value*
Parathyroid hormone, mean (s.d.), mg dl^{-1}	113 (80)	230 (105)	0.001
Alkaline phosphatase, mean (s.d.), U l^{-1}	417 (169)	456 (205)	0.59
Serum calcium, mean (s.d.), mg dl^{-1}	9.3 (0.9)	9.1 (0.7)	0.60
Serum phosphorus, mean (s.d.), mg dl^{-1}	5.8 (1.2)	4.6 (1.2)	0.031

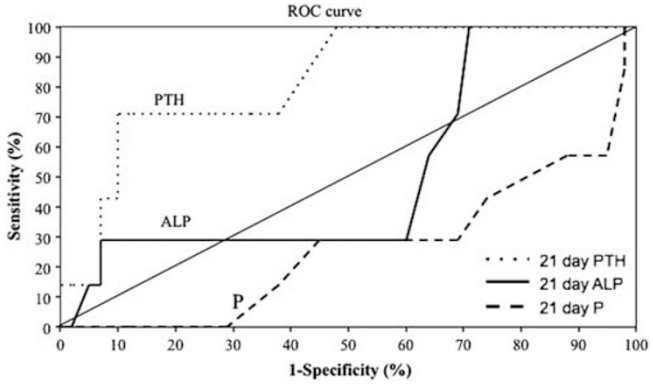

Figure 2. ROC curve of PTH, ALP, P for rickets.

Calcium carbonate data

Three infants (43%) with severe MBD and thirty-three infants (79%) in the mild osteopenia cohort were treated with $CaCO_3$ (average of 5 ± 9 days and 9 ± 8 days, respectively) before their 6-week knee x-ray.

Morbidity and medication outcomes

Infants with severe MBD had significantly higher incidences of periventricular leukomalacia, treated retinopathy of prematurity and patent ductus arteriosus, spontaneous intestinal perforation and had a longer hospital stay ($P < 0.05$) (Table 5). Patients with severe osteopenia also had more steroid use (21 days vs 4 days) and exposure to caffeine (64 days vs 43 days).

We examined variables known to be associated with MBD and variables that were significant on univariate analysis (birth weight, gender, intrauterine growth restriction, 21-day PTH and P, Ca and P intake, steroid and caffeine use, periventricular leukomalacia, retinopathy of prematurity, patent ductus arteriosus and spontaneous intestinal perforation) to examine independent correlates and their effects on severe MBD. To determine whether the PTH level could predict severe MBD at 6 weeks of age, we entered variables which would affect bone metabolism in the first 3 weeks of life. The model consisted of birth weight, intrauterine growth restriction, P at 21 days, and for clinical applicability we dichotomized PTH levels (as greater than or less than 180 mg dl^{-1}) at 21 days and also dichotomized the use of postnatal steroids (yes/no). After performing the multivariate logistic regression, PTH level at 21 days remained a significant risk factor for severe MBD (odd's ratio: 22.9; 95% confidence interval: 1.8, 294).

DISCUSSION

Secondary hyperparathyroidism is a common finding in very preterm neonates and has been associated with decreased bone mineralization.[32] The present study demonstrates PTH as an important serologic marker that can be used to detect severe MBD on lower extremity radiographs at 6 weeks and is a better screening tool than ALP. More specifically, a PTH level exceeding 180 mg dl^{-1} at 3 weeks of age should alert clinicians that

Table 3. Diagnostic accuracy of 21-day biochemical marker for severe MBD

Marker	*Maximum on ROC curve*	*Area under ROC curve*	*Sensitivity, %*	*Specificity, %*	*Negative predictive value, %*	*Positive likelihood ratio*
PTH	180 mg dl^{-1}	81	71	88	95	6
ALP	660 U l^{-1}	49	29	93	89	4
PTH >180 mg dl^{-1} or Phosphorus <4.6 mg dl^{-1}			100	94	100	17
ALP>660 U l^{-1} or Phosphorus <4.6 mg dl^{-1}			43	88	90	4

Abbreviations: ALP, alkaline phosphatase; PTH, parathyroid hormone.

Table 4. Nutritional data

Metabolic bone disease	*Mild,* n = *42*	*Severe,* n = *7*	P-*value*
Days to full feeds, mean (s.d.)	31 (17)	43 (20)	0.09
Days to regain birth weight, mean (s.d.)	10 (4)	11 (5)	0.50
Total parenteral nutrition days, mean (s.d.)	25 (12)	33 (14)	0.09
Average 21-day weight gain, mean (s.d.), g kg^{-1} per day	11 (6)	13 (7)	0.56
21-Day calcium intake, mean (s.d.) mg kg^{-1} per day	60 (23)	57 (22)	0.79
21-Day phosphorus intake, mean (s.d.), mg kg^{-1} per day	37 (17)	36 (16)	0.98
21-Day enteral intake, mean (s.d.), kcal kg^{-1}per day	27 (17)	20 (24)	0.38
21-Day parenteral intake, mean (s.d.), kcal kg^{-1}per day	52 (20)	64 (18)	0.16

Table 5. Morbidity and medication outcomes

Metabolic bone disease	*Mild,* n = *42*	*Severe,* n = *7*	P-*value*
Extra-uterine growth restriction (%)	52	86	0.21
Bronchopulmonary disease (%)	21	29	0.65
Intraventricular hemorrhage >grade II (%)	5	43	0.02
Periventricular leukomalacia (%)	10	57	0.02
Retinopathy of prematurity, treated (%)	9	31	0.01
Necrotizing enterocolitis (%)	14	14	1.00
Spontaneous intestinal perforation (%)	0	29	0.02
Patent ductus arteriosus, treated (%)	12	57	0.02
Length of hospital stay, mean (s.d.), days	83 (23)	111 (31)	0.007
Steroid days, mean (s.d.)	4 (9)	21 (21)	0.001
Diuretic days, mean (s.d.)	2 (6)	4 ± 5	0.36
Caffeine days, mean (s.d.)	43 (21)	64 (17)	0.01

nutritional supplementation, radiologic imaging and close follow-up is recommended.

The PTH signaling pathway is well-studied in human adults.[16,18,33,34] These studies suggest that PTH activates the surface receptor activator of nuclear factor kappa B ligand (RANKL) on osteoblasts to bind with RANK on osteoclasts. This fusion of receptor and ligand results in stimulation of osteoclasts and consequently mobilization of Ca from the skeleton. This sequence of events leads to bone demineralization; however, the body's natural response to osteoclastogenesis is to form new bone.[35] ALP is membrane-bound on the osteoblast and transports P into the cell to allow merger with Ca.[20] Our justification for the use of PTH is that this hormone regulates the function of osteoblasts and therefore directly influences ALP. Moreover, our concern for using tissue non-specific ALP as a marker for MBD is based on the multiple tissues that affect ALP levels.[36–39]

This study supports the findings of Mitchell *et al.*[40] which state that MBD and elevated serum ALP levels continue to exist in very preterm infants. ALP is currently the most frequently used serum marker to evaluate bone mineralization; however, its ability to predict changes in bone metabolism has become controversial. Hung *et al.*,[41] found that ALP levels exceeding 700 U l^{-1}, at 3 weeks of age, resulted in a sensitivity and specificity of 73% in predicting MBD in premature infants. Although comparable to our predictive values, an important distinction from their study is that they defined osteopenia according to Koo's criteria, which includes thinning of cortex and loss of dense line at the metaphysis (our equivalent of mild MBD).[26] In 2010, Taylor *et al.*[42] also looked at ALP as a screening test for MBD and although their ALP cutoff values coincided with ours, they had a higher specificity (94% vs 88%), but their patient population had a mean age of 1 year and imaging was only performed in patients who demonstrated an elevated ALP. Levels of ALP >900 U l^{-1} in conjunction with P levels < 4.6 mg dl^{-1} at 3 months of age have correlated with MBD in premature neonates.[22]

Although the 3-week Ca levels were similar between the two groups, the infants with severe MBD had a slightly lower level. In turn, the P was significantly lower in the patients with severe MBD. Our study population was obtaining comparable rates of these bone-forming minerals; half the rate typically accrued *in-utero*.[5] Therefore, one would expect the P levels to also be similar among the two cohorts. This outcome strengthens the argument that secondary hyperparathyroidism is a physiologic adjustment premature neonates experience to normalize extracellular Ca. As PTH continues to rise the serum P decreases secondary to its phosphaturic effect on the kidney.

Many factors have been linked to the development of MBD. For example, corticosteroid, caffeine and diuretic use have been associated with osteopenia.[43–45] Our patients were rarely exposed to diuretics and thus, did not have a significant role in our study. On the other hand, our results were in agreement with Eello and colleagues[43] who emphasize the deleterious effects corticosteroids have on bone mineralization. Glucocorticoids inhibit bone formation by increasing cell death of osteoblasts and osteocytes, as well as supporting osteoclast differentiation.[46,47] Additionally, similar to glucocorticoids, studies have shown that caffeine adversely affects intestinal absorption of Ca and stimulates urinary excretion of Ca.[44,48] An important factor in bone metabolism is overall neonatal health. Not surprising, the cohort of infants with severe MBD were sicker infants, represented by longer hospital stay and increase in morbidities (spontaneous intestinal perforation, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity, patent ductus arteriosus).

Another factor that may induce MBD and that has recently gained much pediatric attention is vitamin D deficiency. However, in a study from Poland, term newborns with vitamin D deficiency did not elicit elevated concentrations in PTH nor ALP.[49] Correspondingly, Abrams[10] describes most cases of osteopenia in very low birth weight infants is not secondary to vitamin D deficiency but instead secondary to a lack of Ca and P accretion *ex-utero*. The majority of Ca and P absorption in the premature gut occurs passively and does not require vitamin D dependent active transport.[50] Animal studies validate these findings demonstrating that severe MBD and even death can occur in the absence of parathyroid glands and PTH, while absence of vitamin D does not significantly interrupt serum Ca or P.[51] In our development of our unit's policy and guidelines, vitamin D was measured in a series of infants and was within normal limits within the first month of life (data not included). Additionally, the current nutritional guidelines from the American Academy of Pediatrics recommends very low birth weight neonates to receive a daily amount of 200-400 IU of vitamin D.[10] Our patient population was receiving 140 IU of vitamin D simply from their parenteral nutrition.

A major limitation to this study includes the use of lower extremity radiographs to assess bone mineralization, as studies have shown that at least 20% of demineralization must occur before being captured on x-ray.[25] Although x-ray detection of MBD is flawed, it is the most commonly used and studied imaging technique in neonatal bone research. We chose to use this method for its clinical applicability, its results are generalizable (as most NICUs have access to x-ray), and it was the technique available at our institution. Another drawback to this study is the subjectivity in the interpretation of the knee radiographs given that all of our patients had some degree of MBD. The present-day 'gold standard' for bone mineral content and density is dual-energy x-ray absorptiometry but it is not in current widespread use and remains primarily an investigative tool. A limitation to the routine use of PTH sampling is the higher expense (~3 times) when compared with ALP testing (courtesy of ARUP Laboratories, Salt Lake City, UT, USA).

Early PTH may quickly identify neonates at risk for MBD and be a useful marker for monitoring infants with a birth weight < 1250 g. Future studies should attempt to correlate PTH values with newer imaging techniques, such as quantitative ultrasonography and dual-energy X-ray absorptiometry.

CONCLUSION

In summary, this study provides evidence that the use of PTH is an early serum biomarker in detecting MBD. At present, our understanding of bone metabolism and the development of MBD in premature neonates is limited and understudied. New venues need to be explored in neonatal nutrition in attempts to decrease the short and long-term complications of MBD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Lothe A, Sinn J, Stone M. Metabolic bone disease of prematurity and secondary hyperparathyroidism. *J Paediatr Child Health* 2011; **47**(8): 550–553.
2 Chan GM, Armstrong C, Moyer-Mileur L, Hoff C. Growth and bone mineralization in children born prematurely. *J Perinatol* 2008; **28**(9): 619–623.
3 Lucas-Herald A, Butler S, Mactier H, McDevitt H, Young D, Ahmed SF. Prevalence and characteristics of rib fractures in ex-preterm infants. *Pediatrics* 2012; **130**(6): 1116–1119.
4 Viswanathan S, Khasawneh W, McNelis K, Dykstra C, Amstadt R, Super DM *et al.* Metabolic bone disease: a continued challenge in extremely low birth weight infants. *JPEN J Parenter Enteral Nutr;* e-pub ahead of print 2013; doi:10.1177014860711349959O
5 Rigo J, Pieltain C, Salle B, Senterre J. Enteral calcium, phosphate and vitamin D requirements and bone mineralization in preterm infants. *Acta Paediatr* 2007; **96**(7): 969–974.
6 Harrison CM, Johnson K, McKechnie E. Osteopenia of prematurity: a national survey and review of practice. *Acta Paediatr* 2008; **97**(4): 407–413.
7 Lucas A. Long-term programming effects of early nutrition -- implications for the preterm infant. *J Perinatol* 2005; **25**(Suppl 2): S2–S6.
8 Cooper C, Westlake S, Harvey N, Dennison E. Developmental Origins of Osteoporotic Fracture In: Goldberg G, Prentice A, Prentice A, Filteau S, Simondon K (eds) *Breast-Feeding: Early Influences on Later Health*. Springer: Netherlands, 2009, pp 217–236.
9 Wood CL, Wood AM, Harker C, Embleton ND. Bone mineral density and osteoporosis after preterm birth: the role of early life factors and nutrition. *Int J Endocrinol*; 2013; e-pub ahead of print 2013; doi:10.1155/2013/902513.
10 Abrams SA, Bhatia JJS, Abrams SA, Corkins MR, Ferranti SD, de, Golden NH *et al.* Calcium and Vitamin D Requirements of Enterally Fed Preterm Infants. *Pediatrics* 2013; **131**(5): e1676–e1683.
11 Salle BL, David L, Chopard JP, Grafmeyer DC, Renaud H. Prevention of early neonatal hypocalcemia in low birth weight infants with continuous calcium infusion: effect on serum calcium, phosphorus, magnesium, and circulating immunoreactive parathyroid hormone and calcitonin. *Pediatr Res* 1977; **11**(12): 1180–1185.
12 Pitkin RM, Cruikshank DP, Schauberger CW, Reynolds WA, Williams GA, Hargis GK. Fetal calcitropic hormones and neonatal calcium homeostasis. *Pediatrics* 1980; **66**(1): 77–82.
13 Venkataraman PS, Blick KE, Fry HD, Rao RK. Postnatal changes in calcium-regulating hormones in very-low-birth-weight infants. Effect of early neonatal hypocalcemia and intravenous calcium infusion on serum parathyroid hormone and calcitonin homeostasis. *Am J Dis Child* 1985; **139**(9): 913–916.
14 Rubin LP, Posillico JT, Anast CS, Brown EM. Circulating levels of biologically active and immunoreactive intact parathyroid hormone in human newborns. *Pediatr Res* 1991; **29**(2): 201–207.
15 Cooper LJ, Anast CS. Circulating immunoreactive parathyroid hormone levels in premature infants and the response to calcium therapy. *Acta Paediatr* 1985; **74**(5): 669–673.
16 Huang JC, Sakata T, Pfleger LL, Bencsik M, Halloran BP, Bikle DD *et al.* PTH differentially regulates expression of RANKL and OPG. *J Bone Miner Res* 2004; **19**(2): 235–244.
17 Khosla S. Minireview: the OPG/RANKL/RANK system. *Endocrinology* 2001; **142**(12): 5050–5055.
18 Swarthout JT, D'Alonzo RC, Selvamurugan N, Partridge NC. Parathyroid hormone-dependent signaling pathways regulating genes in bone cells. *Gene* 2002; **282**(1-2): 1–17.
19 Bozzetti V, Tagliabue P. Metabolic bone disease in preterm newborn: an update on nutritional issues. *Ital J Pediatr* 2009; **35**(1): 20.
20 Tinnion RJ, Embleton ND. How to use. alkaline phosphatase in neonatology. *Arch Dis Child Educ Pract Ed* 2012; **97**(4): 157–163.
21 Golub EE, Harrison G, Taylor AG, Camper S, Shapiro IM. The role of alkaline phosphatase in cartilage mineralization. *Bone Miner* 1992; **17**(2): 273–278.
22 Backström MC, Kouri T, Kuusela AL, Sievänen H, Koivisto AM, Ikonen RS *et al.* Bone isoenzyme of serum alkaline phosphatase and serum inorganic phosphate in metabolic bone disease of prematurity. *Acta Paediatr* 2000; **89**(7): 867–873.
23 Faerk J, Peitersen B, Petersen S, Michaelsen KF. Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. *Arch Dis Child Fetal Neonatal Ed* 2002; **87**(2): F133–F136.
24 Visser F, Sprij AJ, Brus F. The validity of biochemical markers in metabolic bone disease in preterm infants: a systematic review. *Acta Paediatr* 2012; **101**(6): 562–568.
25 Done SL. Fetal and neonatal bone health: update on bone growth and manifestations in health and disease. *Pediatr Radiol* 2012; **42**(Suppl 1): S158–S176.
26 Koo WW, Tsang R. Bone mineralization in infants. *Prog Food Nutr Sci* 1984; **8**(3-4): 229–302.
27 Shore RM, Chesney RW. Rickets: part II. *Pediatr Radiol* 2013; **43**(2): 152–172.
28 Catache M, Leone CR. Role of plasma and urinary calcium and phosphorus measurements in early detection of phosphorus deficiency in very low birthweight infants. *Acta Paediatr* 2003; **92**(1): 76–80.
29 Harrison CM, Gibson AT. Osteopenia in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 2013; **98**(3): F272–F275.
30 Vachharajani AJ, Mathur AM, Rao R. Metabolic bone disease of prematurity. *NeoReviews* 2009; **10**(8): e402–e411.
31 Moreira A, Caskey M, Fonseca R, Malloy M, Geary C. Impact of providing vitamin A to the routine pulmonary care of extremely low birth weight infants. *J Matern Fetal Neonatal Med* 2012; **25**(1): 84–88.
32 Moreira A, February M, Geary C. Parathyroid hormone levels in neonates with suspected osteopenia. *J Paediatr Child Health* 2013; **49**(1): E12–E16.
33 Kraenzlin ME, Meier C. Parathyroid hormone analogues in the treatment of osteoporosis. *Nat Rev Endocrinol* 2011; **7**(11): 647–656.
34 Buenzli PR, Pivonka P, Gardiner BS, Smith DW. Modelling the anabolic response of bone using a cell population model. *J Theor Biol* 2012; **307**: 42–52.
35 Redlich K, Görtz B, Hayer S, Zwerina J, Doerr N, Kostenuik P *et al.* Repair of local bone erosions and reversal of systemic bone loss upon therapy with anti-tumor necrosis factor in combination with osteoprotegerin or parathyroid hormone in tumor necrosis factor-mediated arthritis. *Am J Pathol* 2004; **164**(2): 543–555.
36 Garattini E, Margolis J, Heimer E, Felix A, Udenfriend S. Human placental alkaline phosphatase in liver and intestine. *Proc Natl Acad Sci USA* 1985; **82**(18): 6080–6084.
37 Islam MT, Islam MN, Mollah AH, Hoque MA, Hossain MA, Nazir F *et al.* Status of liver enzymes in babies with perinatal asphyxia. *Mymensingh Med J* 2011; **20**(3): 446–449.
38 Ferianec V, Linhartová L. Extreme elevation of placental alkaline phosphatase as a marker of preterm delivery, placental insufficiency and low birth weight. *Neuro Endocrinol Lett* 2011; **32**(2): 154–157.
39 Van Hoof VO, De Broe ME. Interpretation and clinical significance of alkaline phosphatase isoenzyme patterns. *Crit Rev Clin Lab Sci* 1994; **31**(3): 197–293.
40 Mitchell SM, Rogers SP, Hicks PD, Hawthorne KM, Parker BR, Abrams SA. High frequencies of elevated alkaline phosphatase activity and rickets exist in extremely low birth weight infants despite current nutritional support. *BMC Pediatr* 2009; **9**: 47.
41 Hung Y-L, Chen P-C, Jeng S-F, Hsieh C-J, SS-F Peng, Yen R-F *et al.* Serial measurements of serum alkaline phosphatase for early prediction of osteopaenia in preterm infants. *J Paediatr Child Health* 2011; **47**(3): 134–139.
42 Taylor JA, Richter M, Done S, Feldman KW. The utility of alkaline phosphatase measurement as a screening test for rickets in breast-fed infants and toddlers: a study from the puget sound pediatric research network. *Clin Pediatr* 2010; **49**(12): 1103–1110.
43 Eelloo JA, Roberts SA, Emmerson AJB, Ward KA, Adams JE. Mughal MZ. Bone status of children aged 5-8 years, treated with dexamethasone for chronic lung disease of prematurity. *Arch Dis Child Fetal Neonatal Ed* 2008; **93**(3): F222–F224.
44 Zhou Y, Guan XX, Zhu ZL, Guo J, Huang YC, Hou WW *et al.* Caffeine inhibits the viability and osteogenic differentiation of rat bone marrow-derived mesenchymal stromal cells. *Br J Pharmacol* 2010; **161**(7): 1542–1552.
45 Rowe JC, Carey DE, Goetz CA, Adams ND, Horak E. Effect of high calcium and phosphorus intake on mineral retention in very low birth weight infants chronically treated with furosemide. *J Pediatr Gastroenterol Nutr* 1989; **9**(2): 206–211.
46 Yao W, Dai W, Jiang JX, Lane NE. Glucocorticoids and osteocyte autophagy. *Bone* 2013; **54**(2): 279–284.
47 Weinstein RS. Glucocorticoid-induced osteonecrosis. *Endocrine* 2012; **41**(2): 183–190.
48 Shrivastava A, Lyon A, McIntosh N. The effect of dexamethasone on growth, mineral balance and bone mineralisation in preterm infants with chronic lung disease. *Eur J Pediatr* 2000; **159**(5): 380–384.
49 Czech-Kowalska J, Pludowski P, Dobrzanska A, Kryskiewicz E, Karczmarewicz E, Gruszfeld D *et al.* Impact of vitamin D supplementation on markers of bone mineral metabolism in term infants. *Bone* 2012; **51**(4): 781–786.
50 Koo WWWK, Warren L. Calcium and bone health in infants. *Neonatal Netw* 2003; **22**(5): 23–37.
51 Kovacs CS. Bone development in the fetus and neonate: role of the calciotropic hormones. *Curr Osteoporos Rep* 2011; **9**(4): 274–283.