Role of Biochemical Parameters for The Detection of Osteopenia of Prematurity

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Abstract

Background: Complications from preterm birth are the leading causes of death among children under 5 years of age, responsible for approximately 1 million deaths in 2015. Three-quarters of these deaths could be prevented with current, cost-effective interventions. Premature infants, particularly those born at < 28 weeks of gestation, are at significant risk for reduced bone mineral content (BMC) and subsequent bone disease, variably termed metabolic bone disease (MBD), osteomalacia, osteopenia, or neonatal rickets. Risk of fracture and growth failure increase in the presence of osteopenia in these infants. Early detection of Osteopenia of Prematurity (OOP) may prevent unwanted deaths.

Objectives: To identify biochemical markers of osteopenia in the neonatal period for early detection before the appearance of radiological evidence of osteopenia.

Methods: This prospective observational study was carried out at Dhaka Shishu Hospital (DSH) from July, 2016 to June, 2018. Babies admitted in the Neonatal Intensive Care and Special Care Baby Unit with gestational age < 34 weeks and birth weight < 2200 gm were included in the study. Purposive sampling technique was used to collect the study data. Blood sample for baseline biochemical markers were collected in 1st week of life, then subsequently at 2 weeks interval up to corrected term age. Wrist radiography was done for to detect radiological osteopenia at 6th week post-natal age and at corrected term age. Then the biochemical parameters are compared with radiological osteopenia.

Result: This study included 84 preterm new born below 34 weeks of gestation and a birth weight below 2200 grams. Radiological evidence of osteopenia of prematurity was found in 34 (40.5%) babies of which 29 (85.3%) were below 32 week and 5 (14.7%) were at or above 32 weeks. Osteopenic infants had significantly ($p < 0.001$) lower birth weight (1318.82 ± 264.23 gm) compared to non-osteopenic infants (1701.40 ± 431.11 gm). Our study showed that the optimal cutoff point for Alkaline phosphatase (ALP) was 352.50 U/L at 3rd week of life, at which sensitivity and specificity were found 82.4% and 80.0% respectively. Serum inorganic phosphate values for the diagnosis of osteopenia was 4.67 mg/dl at 5th week of age. At this level, sensitivity was found to be 68.0%, whereas specificity was 60.0%. Serum calcium of the infants did not show any significant difference when they were enrolled in the study and in the first follow up. But serum calcium level was significantly lower in osteopenic infants compared to non-osteopenic infants in 2nd and 3rd follow up ($p < 0.05$).

Conclusion: High alkaline phosphatase level at 3rd week of life and low serum inorganic phosphate at 5th week of life can be used as a predictor of osteopenia of prematurity.

Keywords: Osteopenia of Prematurity, Metabolic Bone Disease of Prematurity, Biomarkers, Rickets, Alkaline Phosphatase, Inorganic phosphate.

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Introduction

Osteopenia of prematurity, synonymously metabolic bone disease (MBD) of prematurity, is an important comorbidity often encountered in premature and low birth weight babies.[1] As per the studies, Bangladesh ranks 7th among the top 10 countries with the greatest number of preterm birth.[2] The condition worsens if it is associated with other complications and comorbidities. The occurrence of osteopenia is one of the common complications among low birth weight and preterm babies, which might lead to growth retardation, delayed bone maturation and risk of fractures. Most previous studies reported that incidences of osteopenia of prematurity (OOP) were as high as up to 75%.[3,4] A recent study showed that the prevalence of OOP in two specialty centers in

Bangladesh is about 28%.[5] And The complications of osteopenia can be reduced to a greater extent if useful biochemical markers can be detected.

Early studies showed that fractures in premature infants typically occur several weeks after delivery and before the postnatal age of 6 months, with an incidence varying between 2.1 and 25%.[6,7] Contemporary diagnosis of overt osteopenia has been based on characteristic radiological changes in the skeletal system.[8] However, the radiological changes of osteopenia are not easily detected until bone mineralization is reduced by at least 20%.[9]

Identification of reliable biochemical markers of osteopenia in the early neonatal period is essential to detect osteopenia early and to prevent consequences. This study finding can help defining serum levels of biochemical markers of osteopenia in premature infants.

Our objective was to measure serum alkaline phosphatase, serum calcium and serum inorganic phosphate level of preterm infants at 1st, 3rd and 5th weeks of postnatal age in early detection of osteopenia of prematurity. More precisely to detect radiologic evidence of osteopenia of prematurity at 6th week postnatal age and at corrected term age and to correlate radiologic osteopenia in premature infants with serum levels of biochemical markers like serum calcium, inorganic phosphate and alkaline phosphatase.

Materials and Methods

It was a Prospective Observational Study carried out in the Department of Neonatology, Dhaka Shishu Hospital, from July 2016 to June 2018. Preterm infants admitted in the Neonatal Intensive Care and Special Care Baby Unit of Dhaka Shishu Hospital were included in the study on the basis of the following **inclusion criteria**

a) babies born at <34 weeks of Gestation

b) babies with birth weight 2200g.

exclusion criteria

a) who were hemodynamically unstable premature,

b) who had congenital anomalies

c) suffering from cholestatic jaundice and

d) neonates with syndromic manifestations or suspected inborn errors of metabolism.

Ethical clearance and permission had been taken from the Ethical Review Committee of Bangladesh Institute of Child Health, Dhaka, Bangladesh. Informed written consent was taken from the patient or patients' guardian after duly informing the procedure of treatment, anticipated result, possible advantages, disadvantages and complications considering all ethical issues.

Study Procedure

Hospitalized out-born babies admitted within 3 days of life were included consecutively. Infants' baseline demographics including gestational age (GA), birth weight (BW), small for gestational age (SGA) (by using Fenton preterm growth chart for boys and girls), gender and perinatal characteristics including place of delivery, mode of delivery, APGAR score at 5 minutes (if available from the referral sheet), and maternal characteristics including gravida, parity, hypertension, diabetes mellitus, presence of risk factors of sepsis (PROM, UTI etc.) were collected from history. Postnatal events including respiratory distress syndrome, sepsis, time to full feeding were recorded.

Blood samples for biochemical tests were done biweekly from 1 (one)-week postnatal age onwards up to corrected term age. During study period, these biochemical markers were incorporated into routine biochemical follow-up of premature infants and sample was taken aseptically adjusting with other purposes of venipuncture. Frequency of biochemical testing was done according to the infants' gestational age. Frequency was at least 3 in infants with gestational age < 32 weeks, 4 in < 31 weeks.

Radiologic examination of forearm of enrolled premature infants was done with X-ray machine at 6th week postnatal age. Patients' outcomes including presence of osteopenia, length of hospital stay, gestational age at radiologic examination, gestational age at discharge were documented. We have considered scoring method (Koo's scoring) using an antero-posterior radiograph of the right forearm and wrist.[8] Grading of radiologic findings of osteopenia of prematurity are as follows:

i. **Grade 0 (Normal):** Normal density of bony cortex along shaft with normal dense white line at metaphyses and a normal band of lucency in the submetaphyseal region

ii. **Grade 1:** Mineral rarefaction characterized by loss of dense white line at metaphyses, increased sub metaphyseal lucency and thinning of the cortex.

iii. **Grade 2:** Changes of grade 1 plus irregularity, fraying and cupping of the metaphyses- characteristic findings of rickets.

iv. **Grade 3:** Changes of rickets with evidence of fractures.

Osteopenia is considered when there is radiographic evidence of diminished bone density defined as > grade 1 in Koo's score.

All infants received parenteral nutrition with 5-10% dextrose in aqua in the first 24 hours and 5-10% dextrose with electrolytes was introduced after 24 hours of age. Enteral feeding was introduced and advanced with breast milk as per existing institutional guideline. Full enteral feeding was targeted at 150 ml/kg/day. Parenteral intravenous fluid was stopped after

achieving full enteral feeding. Vitamin and mineral supplementation were prescribed as per institutional policy. Hypocalcemia was managed accordingly.

A study flow chart (Figure 1) is mentioned here which was partially adapted from Rustico et al.[10]

differences of biochemical parameter and for continuous variables between two groups. Receiver operator characteristics (ROC) curves were used to determine the individual diagnostic performance of serum alkaline phosphatase and serum inorganic phosphate at 3 weeks postnatal age in predicting the occurrence of osteopenia at corrected term age.

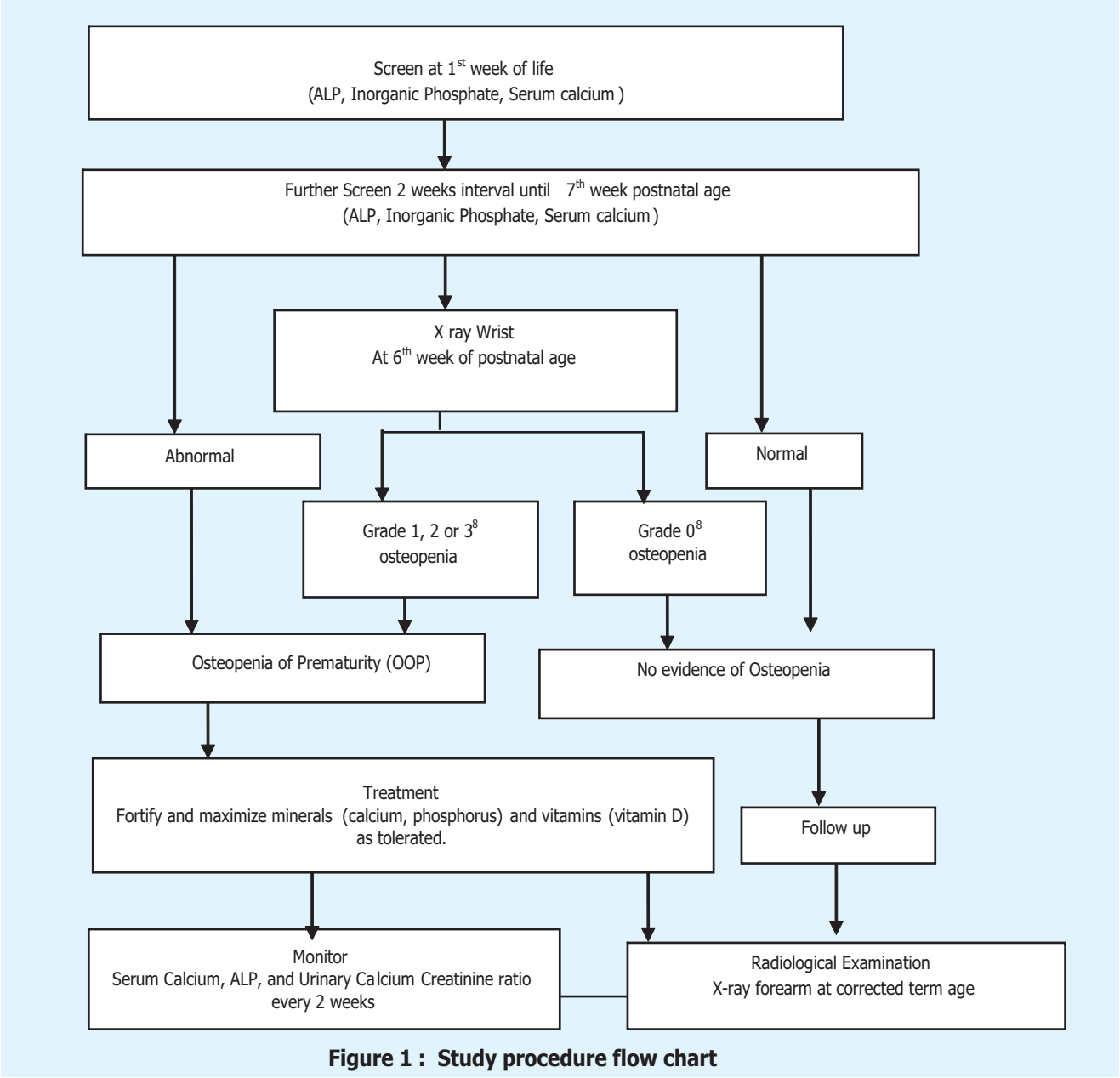

Figure 1 : Study procedure flow chart

All quantitative data were expressed as the mean ± standard deviation and categorical data were presented as frequency. Demographic, perinatal variables, clinical and outcome were compared between two groups (osteopenic and non-osteopenic) using Chi Squared test for categorical variables and Independent-t test were applied to calculate the significance in

The statistical software IBM SPSS Statistics version 22 (SPSS Chicago, IL, USA) was used for the statistical analysis of the data. Results were considered statistically significant at p value < 0.05.

Results

Among the 130 enrolled babies, 84 completed all 3 follow up visits.

Final analysis was done with the data of 84 babies.

Demographic variables are shown in Table I. The enrolled neonates were admitted at around 1.88 post-natal days with an average gestational age of 31.2 weeks. Fifty-seven percent neonate were male. Regarding mode of delivery 82.1% babies born per vaginally. Regarding the maternal characteristics, mother of 54.76% admitted newborn were multiparous, 33.33% mother having infection during the pregnancy period, 10.71% mother experienced gestational hypertension and only one mother had Gestational diabetes.

Table I : Demographic variables

Variables	Value/ Frequency
1. Baseline Characteristics of Enrolled Infants	
Age at admission (in days) (mean ± SD)	1.88 ± 0.99
Gestational age (in weeks) (mean ± SD)	31.20 ± 1.75
Weight at admission (in grams) (mean ± SD)	1532.62 ± 393.89
2. Sex	
Male	48 (57.1%)
Female	36 (42.9%)
3. Mode of delivery	
NVD	69 (82.1%)
Other Than NVD	15 (17.9%)
4. Maternal Characteristics	
Multiparity	46 (54.76%)
Maternal infection	28 (33.33%)
Gestational hypertension	9 (10.71%)
Gestational diabetes	1 (1.20%)

In this study, it is evident that 40.5% newborns showed radiological evidence of osteopenia. Among them, significant number of newborn (85.3%) with a gestational age below 32 weeks had the evidence of radiological osteopenia, whereas only 14.7% newborn with a gestational age beyond 32 weeks had radiological osteopenia (Table II), which is statistically significant. Osteopenic infants had significantly lower birth weight (1318.82 ± 264.23 gm) compared to non-osteopenic infants (1701.40 ± 431.11 gm) (p<0.001).

Table II: Presence of radiologic evidence of osteopenia (at 6th week postnatal age) according to gestational age

Variable	Presence of Osteopenia No. (%)	Absence of Osteopenia No. (%)	p- value
< 32 weeks gestation	29 (85.3%)	15 (30.0%)	<0.001
32 weeks gestation	5 (14.7%)	35 (70.0%)	

Figure 2 demonstrates the radiological grading of osteopenia. Grade 1 osteopenia was about 4 times higher 40.9% vs 10%). Similarly Grade 2 osteopenia was found 10 times higher (25% vs 2.5%) among the newborn with gestational age below 32 weeks. We found no patient with Grade 3 or severe osteopenia among the groups. It reflects that premature infant with gestational age below 32 weeks had remarkable number of osteopenia than in newborn with GA above 32 weeks.

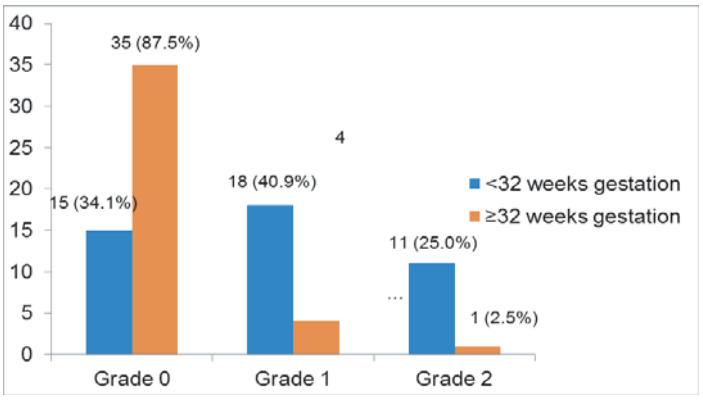

Figure 2. Grading of osteopenia according to gestational age

We found no statistical significance with the co-morbidities among the osteopenic and non-osteopenic neonates. The co-morbidities include RDS, Sepsis, Necrotizing Enterocolitis, and need for mechanical Ventilation.

Our point of interest is the values of biochemical markers of osteopenia are tabulated in Table III. It shows the level of serum alkaline phosphatase, serum inorganic phosphate and serum calcium levels of infants. Serum alkaline phosphatase of the infants did not show any significant difference when they were enrolled in the study. But serum alkaline phosphatase level was significantly higher in osteopenic infants compared to non-osteopenic infants in every follow up (p<0.05).

The level of serum inorganic phosphate of the infants (showed in table IIIb). Serum inorganic phosphate of the infants did not show any significant difference during enrollment. But serum inorganic phosphate level was significantly higher in osteopenic infants compared to non-osteopenic infants in every follow up (p<0.05). The level of serum calcium of the infants (showed in table IIIc). Serum calcium of the infants did not show any significant difference when they were enrolled in the study and in the first follow up. But serum calcium level was significantly lower in osteopenic infants compared to non-osteopenic infants in second and third follow up (p<0.05).

Table III : Values of serum alkaline phosphatase, serum inorganic phosphatase and serum calcium level among the osteopenic and non-osteopenic neonates in chronological follow-up

	a) Serum alkaline phosphatase (IU/L)			b) Serum inorganic phosphate (mg/dL)			c) Serum calcium (mmol/L)		
	Osteopenic (Mean ± SD)	Non-osteopenic (Mean ± SD)	p value	Osteopenic (Mean ± SD)	Non-osteopenic (Mean ± SD)	p value	Osteopenic (Mean ± SD)	Non-osteopenic (Mean ± SD)	p value
Base line	293.15 ± 22.87	278.60 ±16.99	0.063	4.76 ± 0.22	4.73 ± 0.22	0.54	2.11 ± 0.22	2.13 ± 0.11	0.647
FU-1[a]	394.91 ± 47.76	308.14 ± 21.37	<0.001*	4.44 ± 0.27	4.98 ±0.23	<0.001*	2.06 ± 0.27	2.15 ± 0.11	0.053
FU-2[b]	496.76 ± 60.70	338.90 ± 28.36	<0.001*	3.98 ± 0.38	5.08 ±0.21	<0.001*	1.99 ± 0.28	2.13 ± 0.12	0.013
FU-3[c]	524.62 ± 62.29	362.72 ± 36.93	0.003	3.40 ± 0.54	4.97 ±0.19	<0.001*	1.95 ±0.29	2.11 ± 0.13	0.005

[a]FU-1 : 1st follow up at 3rd week postnatal age, [b]FU-2: 2nd follow up at 5th week postnatal age, [c]FU-3: 3rd follow up at 7th week postnatal age
* Statistically significan

Receiver Operator Characteristics (ROC) curve analysis of serum inorganic phosphate (figure 3) values at 5th week post-natal age for the diagnosis of osteopenia in the present study showed that the optimal cutoff point was determined at the serum inorganic phosphate level of 4.67 mg/dl. At this level, sensitivity was found to be 68.0%, whereas specificity was 60.0%. The area under the curve was 0.944 denoting very high diagnostic accuracy of serum inorganic phosphate in detecting osteopenia.

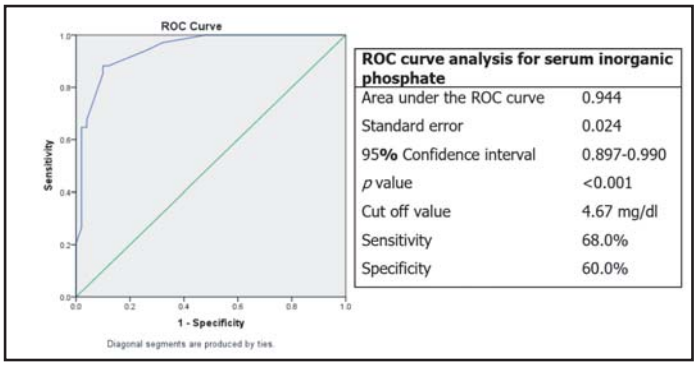

Figure 3. ROC for Inorganic Phosphatase

Receiver Operator Characteristics (ROC) curve analysis of serum alkaline phosphatase (ALP) values (figure 4) at 3rd week of life for the diagnosis of osteopenia in the present study showed that the optimal cutoff point was determined at the ALP level of 352.50 U/L. At this level, sensitivity was found to be 82.4%, whereas specificity was 80.0%. The area under the curve was 0.925 denoting very high diagnostic accuracy of ALP in detecting osteopenia.

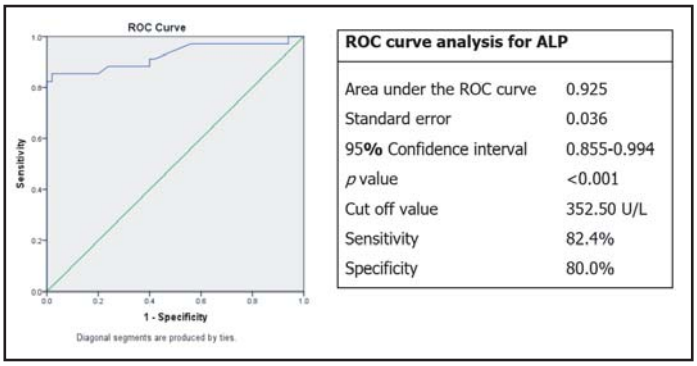

Figure 4. ROC for Alkaline Phosphatase

Discussion

In our study, 40.5% of infants showed radiological evidence of osteopenia at their 6th weeks postnatal age. This result was consistent with the study of You et al. who showed that 40.88% preterm infants were osteopenic.[11] But the prevalence of osteopenia in preterm infants varies country to country. In Spain, the prevalence of osteopenia in preterm infants was found 15.5% and in Cairo it was found 13.3%.[12,13] This variation might be due to the fact that OOP is a multifactorial disease included nutritional factors but also biomechanical and environmental factors.[14]

Most premature infants, especially those born with low birth weight (<1500 g) and young gestational age (<32 weeks' gestation), are at risk for developing osteopenia[15] In the current study, among the infants of <32 weeks of gestation (n=44), most of them (85.3%) were osteopenic whereas among the infants of 32 weeks of gestation (n=40), only 14.7% were osteopenic which showed highly significant statistical difference (p<0.001).

Reduction in bone mineral content and the development of metabolic bone disease of prematurity are quite common among very low birth weight infants.[14] The present study found that osteopenic infants had significantly lower birth weight (1318.82 ± 264.23 gm). This finding was consistent with the findings of Ramón A et al. and Abdallah et al. where they also found that the incidence of osteopenia increases with lower birth weight.[12,13]

Development of respiratory distress syndrome (RDS), need for mechanical ventilation (MV), sepsis and NEC did not show any statistical difference between the groups in the present study.Similar result was found by Abdallah et al. who also did not found any significant statistical difference between osteopenic and non-osteopenic infants regarding development of complication.[13] Ramón A et al. also did not found any significant statistical difference between osteopenic and non-osteopenic infants regarding need for mechanical ventilation.[12]

Measurement of ALP is probably one of the commonest techniques used to assess the likelihood of osteopenia, but there is no clear point at which the levels become diagnostic.[16] At the initial stage, the ALP levels of the infants were 293.15 ± 22.87 U/L and 278.60 ± 16.99U/L in osteopenic and non-osteopenic infants respectively. This difference was statistically significant. ALP rises in all newborns in the first 2-3 weeks of life and increases further if there is insufficient mineral supply, and appropriate mineral supplementation may lead to smaller increases.[16] In the current study, serum ALP level was significantly higher in osteopenic infants compared to non-osteopenic infants in every follow up.

Rustico et al.[10] showed that only the serum ALP level was increased in a fracture group compared to an osteopenia group, and there were no significant differences in serum Phosphate, calcium, or parathyroid hormone (PTH) levels. In contrast, Mitchell et al.[17] found that the ALP level was not significantly higher in ELBW infants with radiologically confirmed rickets compared to those without rickets. Arani et al.[18] also reported no significant differences in ALP levels in VLBW infants between radiologically confirmed rickets/osteopenia and normal groups.

Receiver Operator Characteristics (ROC) curve analysis of serum alkaline phosphatase (ALP) values at 3rd week postnatal showed the optimal cutoff point was 352.50 U/L. At this level, sensitivity was found to be 82.4%, whereas specificity was 80.0%. This result was not consistent with other studies. Other studies found much higher cut off value for ALP for diagnosis of osteopenia which ranged from 473 IU/L to 800 IU/L.[10,12,13,19] This dissimilarity might be due to the different gestational age and birth weight of the study population. Birth weight was significantly inversely related to ALP in the present study, the gestational age ranged from 28 – 34 weeks and birth weight ranged from 1000-2200 gm. But the study of Abdallah et al. reported the cut off value 500IU/L with 100% sensitivity and 80.77% specificity where the birth weight of babies was<1500gm.[13] The study of You et al. reported the cut off value 657.5 IU/L with 81.5% sensitivity and 47.9% specificity where the lowest birth weight of infants was 657.5 gm and lowest gestational age of infant were 24 weeks.[11] Ramón A et al. Reported that the cut off value was 796.5 IU/L with 95.2% sensitivity and 92.4% specificity where the birth weight of babies were <1500gm and gestational below 32 weeks.[12]

Increased ALP and reduced serum Phosphorus have been shown to be related to increased risk of MBD of prematurity.[20] Serum inorganic phosphate of the infants did not show any significant difference among the groups during enrollment. But serum inorganic phosphate level was significantly higher (p<0.05) in osteopenic infants compared to non-osteopenic infants in every follow up.

ROC curve analysis of serum inorganic phosphate values at 5th week post-natal age showed that the optimal cut off point was at the level of 4.67 mg/dl with the sensitivity to be 68.0%, and specificity to be 60.0%. Stacy et al. showed the cut off value for phosphorus for diagnosing osteopenia was <5.5mg/dl.[10] This value was mentioned according to the American Academy of Pediatrics Consensus Statement. The dissimilarity of cutoff value of phosphate in recognizing osteopenia might be due to the fact that the nutritional status of infants does not match between these regions.

In this study, serum calcium of the infants did not show any significant difference during their enrollment and in the first follow up. But serum calcium level was significantly higher (p<0.05) in osteopenic infants in the second follow up (1.99 ± 0.28 mmol/L) and third follow up (1.95 ± 0.29 mmol/L). Serum calcium is not a useful screening test as infants can maintain a normal calcium level at the expense of a loss of bone calcium.[16] So and Ng reported normal calcium level in osteopenic infants which, as they explained, was maintained by parathormone effect that stimulates calcium reabsorption.[21]

Conclusion

Radiologic evidence of Osteopenia was found in 40.5% premature infants and inversely proportional to the gestational age. Lower birth weight is inversely proportionate to development of osteopenia. High alkaline phosphatase level at 3rd week of life and low serum inorganic phosphate at 5th week of life can be used as a predictor of osteopenia of prematurity.

Recommendation

High alkaline phosphatase and low inorganic phosphate levels in preterm infants can be used as screening and monitoring tool for early prediction of osteopenia of prematurity. Further

multicenter studies are required to validate the finding.

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References

1. Viswanathan S, Khasawneh W, McNelis K, et al. Metabolic bone disease: a continued challenge in extremely low birth weight infants. JPEN J Parenter Enteral Nutr 2014; 38: 982–990.
2. Blencowe H, Cousens S, Oestergaard MZ, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379: 2162–2172.
3. Crofton PM, Shrivastava A, Wade JC, et al. Bone and collagen markers in preterm infants: relationship with growth and bone mineral content over the first 10 weeks of life. Pediatr Res 1999; 46: 581–587.
4. Lyon AJ, McIntosh N, Wheeler K, et al. Radiological rickets in extremely low birthweight infants. Pediatr Radiol 1987; 17: 56–58.
5. Afroz N, Chowdhury MA, Hoque MM, et al. Osteopenia in premature infants and effect of supplementation. Bangladesh J Child Health 2015; 39: 135–140.
6. Harrison CM, Johnson K, McKechnie E. Osteopenia of prematurity: a national survey and review of practice. Acta Paediatr 2008; 97: 407–413.
7. Bishop N, Sprigg A, Dalton A. Unexplained fractures in infancy: looking for fragile bones. Arch Dis Child 2007; 92: 251–256.
8. Koo WW, Gupta JM, Nayanar VV, et al. Skeletal changes in preterm infants. Arch Dis Child 1982; 57: 447–452.
9. Ardran GM. Bone destruction not demonstrable by radiography. Br J Radiol 1951; 24: 107–109.
10. Rustico SE, Calabria AC, Garber SJ. Metabolic bone disease of prematurity. J Clin Transl Endocrinol 2014; 1: 85–91.
11. You SK, Lee JE, Lee SM, et al. Metabolic bone disease in preterm infants: Relationship between radiologic grading in the wrist and serum biochemical markers. Diagn Interv Imaging 2017; 98: 785–791.
12. Montaner Ramón A, Fernández Espuelas C, Calmarza Calmarza P, et al. Risk factors and biochemical markers in metabolic bone disease of premature newborns. Rev Chil Pediatr 2017; 88: 487–494.
13. Abdallah EAA, Said RN, Mosallam DS, et al. Serial serum alkaline phosphatase as an early biomarker for osteopenia of prematurity. Medicine (Baltimore) 2016; 95: e4837.
14. Pieltain C, de Halleux V, Senterre T, et al. Prematurity and bone health. World Rev Nutr Diet 2013; 106: 181–188.
15. Diaz R. Osteopenia of Prematurity. In: Brodsky D, Ouellette MA (eds) Primary Care of the Premature Infant. 1600 John F. Kennedy Boulevard Suite 1800 Philladelphia, PA 19103-2899: SAUNDERS ELSEVIER, 2008, pp. 215–221.
16. Harrison CM, Gibson AT. Osteopenia in preterm infants. Arch Dis Child Fetal Neonatal Ed 2013; 98: F272-5.
17. Mitchell SM, Rogers SP, Hicks PD, et al. 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.
18. Arani K, Lotfi A, Jahangiri M, et al. Metabolic Bone Disease in Very Low-Birth-Weight Neonates. IJN 2015; 6: 7–13.
19. You SK, Lee HJ, Park JW, et al. Which serum biochemical markers could predict radiologic metabolic bone disease in preterm infants ? In: Society of Radiology E (ed) Electronic Presentation Online System. Europe: European Society of Radiology, 2016.
20. Aiken CG, Sherwood RA, Lenney W. Role of plasma phosphate measurements in detecting rickets of prematurity and in monitoring treatment. Ann Clin Biochem 1993; 30 (Pt 5): 469–475.
21. So K, Ng P. Treatment and prevention of neonatal osteopenia. Current Paediatrics 2005; 15: 106–113.