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The incidence of osteopenia of prematurity in preterm infants without phosphate supplementation

A prospective, observational study

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Abstract

To meet their requirements for bone mineralization, it is recommended that preterm infants receive nutritional support containing calcium and phosphate. There are no clear data on the incidence of osteopenia of prematurity (OFP) in preterm infants without phosphate supplementation.

This study aimed to investigate the incidence of OFP in preterm infants without phosphate supplementation and its relationship with the duration of parenteral nutrition (PN).

This was a prospective and observational study.

This study included 30 infants aged <32 gestational weeks and weighed <1500 g at birth. All infants received PN according to a standard protocol, beginning on day 1 with calcium, without phosphate. Starting from the first day of life, all infants received human milk without fortifiers. Oral vitamin D (400 IU/d) was administered when enteral nutrition reached 100 mL/kg/d.

The diagnosis of OFP was based on radiographs that were taken of both wrists. Serum alkaline phosphatase (ALP) was measured 3 times: at the start of PN (ALP 1), at the end of PN (ALP 2), and at discharge or the expected due date (ALP 3). Radiographs were obtained on the same day as ALP 3. The duration of PN was analyzed in the presence of OFP using receiver operating characteristic curve analysis.

Among the 30 infants, 13 (43%) were diagnosed with OFP. The duration of PN was significantly longer in the OFP group than in the group without OFP (16 vs 12 days; $P < .05$). The provision of PN for >15 days significantly increased the risk of OFP (odds ratio, 5.40; 95% confidence interval, 1.12–26.04; $P = .035$).

We found a high incidence of OFP in preterm infants without phosphate supplementation. An association was found between the duration of PN and the incidence of OFP. Further research is needed to prevent the development of osteopenia in preterm infants.

Abbreviations: ALP = alkaline phosphatase, BW = birth weight, NEC = necrotizing enterocolitis, NICU = neonatal intensive care unit, OFP = osteopenia of prematurity, PN = parenteral nutrition, PTH = parathyroid hormone, ROC = receiver operating characteristic, SD = standard deviation.

Keywords: osteopenia of prematurity, parenteral nutrition duration, phosphate supplementation

Editor: Antonio Palazón-Bru.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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How to cite this article: Angelika D, Ugrasena ID, Etika R, Rahardjo P, Bos AF, Sauer PJ. The incidence of osteopenia of prematurity in preterm infants without phosphate supplementation: a prospective, observational study. Medicine 2021;100:18(e25758).

Received: 30 April 2020 / Received in final form: 1 March 2021 / Accepted: 9 April 2021

http://dx.doi.org/10.1097/MD.0000000000025758

1. Introduction

Osteopenia of prematurity (OFP) occurs in preterm infants and is characterized by a reduction in bone mineral content (calcium and phosphate). OFP reportedly occurs in 16% to 40% of very low birth weight (BW < 1500 g) infants and in 50% of extremely low birth weight (BW < 1000 g) infants.[1,2] OFP can cause fractures, inadequate weight gain, short stature, and impaired growth. Furthermore, OFP is known to increase the risk of osteoporosis in adulthood.[3] One of the risk factors for OFP is the prolonged use of parenteral nutrition (PN).[4,5] OFP must be detected as early as possible so that appropriate management strategies can be put into place to avoid the development of serious long-term complications.[6]

The American Academy of Pediatrics[7] and the European Society for Paediatric Gastroenterology Hepatology and Nutrition[8] both recommend that PN should be supplemented with calcium and phosphate to meet the requirements for bone mineralization in preterm infants.[7,8] Previous research has demonstrated a link between the incidence of OFP and the provision of PN that does not contain an adequate amount of calcium and phosphate.[9,10] Moreover, the long-term use of PN

is also known to increase the risk of OFP.[4,11] Other research has shown that unfortified human milk has only limited nutritional content and does not provide the amounts of calcium and phosphate that are needed by preterm infants to support their growth.[12,13]

Over the recent years, the use of PN has become increasingly more common in neonatal intensive care units (NICUs) in many countries. The progression of nutritional services for both parenteral and enteral nutrition is known to be affected by the advancement of knowledge and by the availability of supporting nutritional facilities, especially in developed countries. Some countries, however, do not have such facilities; therefore, the progression of nutritional services requires specific improvements, such as phosphate supplementation. To the best of our knowledge, it has yet to be ascertained whether OFP occurs in preterm infants without phosphate supplementation and whether this condition might be related to the duration of PN. Thus, this study aimed to determine the incidence of OFP in preterm infants without phosphate supplementation and its relationship with the duration of PN.

2. Methods

2.1. Patients

All preterm infants[14] aged <32 gestational weeks with a BW <1500 g and a birth date between April 2017 and March 2018 were included in this study. Informed and signed consent was acquired from the parents or authorized representatives of each infant. This study was approved by the Ethical Committee in Health Research at the Dr Soetomo General Hospital, Surabaya (Reference no. 370/Panke.KKE/V/2017). Infants were excluded if they had multiple congenital abnormalities, congenital heart defects with fluid restriction, and asphyxia or if their mothers had diabetes mellitus.[11,15] Additionally, we excluded infants receiving corticosteroid therapy for >2 weeks, furosemide for >2 weeks, and/or aminophylline for >1 month and infants who died before bone mineralization could be measured, infants of parents who did not provide consent, and infants who were withdrawn from the study by their parents.

2.2. Methodology

All infants received PN according to the standard protocol used by our NICU. In brief, PN was administered initially by 4 Fr polyvinyl chloride umbilical catheter (Vygon, Ecouen, France) and subsequently by 1 Fr/28 G peripherally inserted central venous polyurethane catheter (Premicath; Vygon, Ecouen, France). The location of both catheters was controlled by radiography. Since 2012, we have been using all-in-one PN solutions (in which all nutrients are placed in one special bag)[16]; no complications have been reported that were related to this method of administration. PN was provided until infants reached full enteral feeding (150 mL/kg/d). Intravenous amino acids were started on day 1 at a dose of 2 g/kg/d; this was increased daily by 0.5 g/kg/d to 3.5 g/kg/d. We used amino acid solution that contained 6 g/100 mL of amino acids including 9 essential amino acids: histidine, isoleucine, leucine, lysine, valine, methionine, phenylalanine, threonine, and tryptophan (Aminosteril Infant 6%; Fresenius Kabi, Bad Homburg, Germany).[8,17] Urea and creatinine tests were performed every week.[18]

Glucose was provided, along with the amino acids, with an initial glucose infusion rate of 4 to 6 mg/kg/min while maintaining blood glucose levels between 45 and 150 mg/dL. As long as the infant could tolerate it, the glucose infusion rate was increased by 1 mg/kg/min per day. The maximum glucose infusion rate was 12 mg/kg/min. If the glucose level exceeded 200 mg/dL, the rate of glucose infusion was reduced until the glucose level reached the normal range.[8,9]

Lipids were given on the second day, starting with a dose of 1 g/kg/d; this was increased every day by 0.5 g/kg/d to 3 g/kg/d. The lipid solution contained 6% soybean oil, 6% medium chain triglycerides, 5% olive oil, and 3% fish oil (Smoflipid 20%; Fresenius Kabi, Bad Homburg, Germany). Triglyceride tests were performed every week, and triglyceride levels were maintained at <300 mg/dL. If sepsis and cholestasis developed, we reduced the lipid levels until the patient received at least 0.5 g/kg/d of lipid. Lipids were stopped when triglyceride levels exceeded 400 mg/dL.[8,9]

Several electrolytes were provided. We used 2.56 mmol/mL sodium chloride solution (sodium chloride 15%; Pharmaceutical Soetomo Hospital, Surabaya, Indonesia) with a sodium dose of 2 to 4 mmol/kg/d, 1 mmol/mL potassium chloride solution (potassium chloride 7.46%; Otsuka Indonesia, Lawang, Indonesia) with a potassium dose of 1 to 2 mmol/kg/d, and 0.8 mmol/mL magnesium-sulfate solution (magnesium sulfate 20%; Otsuka Indonesia, Lawang Indonesia) with a magnesium dose of 0.1 to 0.3 mmol/kg/d. For calcium, we used 0.25 mmol/mL calcium gluconate solution (calcium gluconate 10%; Otsuka Indonesia, Lawang, Indonesia) with a calcium dose of 0.6 to 1.5 mmol/kg/d. Serum electrolyte tests (sodium, potassium, calcium, and chloride) were carried out every week or as indicated. Neither the phosphate solution nor the trace element solution was available at our hospital; hence, we could not provide both solutions to the infants. Water-soluble vitamin (Soluvit N; Fresenius Kabi, Bad Homburg, Germany) and fat-soluble vitamin (Vitalipid N; Fresenius Kabi, Bad Homburg Germany) were added to the PN solution at doses of 1 mL/kg/d and 4 mL/kg/d, respectively.[8,9]

Minimal enteral nutrition commenced on the first day of life at a dose of 10 mL/kg/d and was increased daily, when tolerated, by 20 mL/kg/d up to a dose of 180 mL/kg/d. Infants received either their own mother's milk or donor milk. No fortifiers were used. When enteral nutrition reached 100 mL/kg/d, we added oral vitamin D at a dose of 400 IU per day.

The diagnosis of OFP was based on radiographs that were taken of both wrists. Radiographs of both wrists were evaluated by an experienced radiologist who was blinded to each patient's clinical details and classified according to the scoring system developed by Koo,[19] as follows. Normal bone density of the bony cortex along the shaft was characterized by a normal dense white line that was present at the metaphysis with a normal band of lucency in the submetaphyseal region. OFP grade 1 was characterized by the disappearance of the normal dense white line at the metaphysis with increased submetaphyseal lucency and thinning of the cortex. OFP grade 2 was characterized by OFP grade 1 changes accompanied by irregularity and fraying of the metaphysis, with splaying and cupping. OFP grade 3 was characterized by changes consistent with rickets, along with evidence of fractures.

Serum alkaline phosphatase (ALP) activity was determined using a colorimetric assay (Dimension EXL; Siemens, Erlangen-Forchheim, Germany), measured 3 times: at the start of PN (ALP

1), at the end of PN (ALP 2), and at discharge or expected due date (ALP 3). Radiographs were obtained on the same day as ALP 3. In our protocol, PN was stopped when the enteral intake volume had reached 150 mL/kg/d.[20] After PN was stopped, the infants received full enteral nutrition until they were discharged from the hospital. Full enteral nutrition was defined as the circumstance in which the infant's overall fluid needs (total of 150–180 mL/kg/d) could be given through the enteral route, either via the orogastric tube or oral feeding. Infants were discharged if they were taking at least 150 mL/kg/d orally, were showing a stable increase in weight, had a minimum weight of 1800 g, were hemodynamically stable, and if the mother could care for the infant with confidence.

2.3. Clinical variables

We collected a range of clinical variables, including gestational age, BW, sex, ALP levels, duration of TPN, sepsis, necrotizing enterocolitis (NEC), patent ductus arteriosus, cholestasis, number of births, and mode of delivery. Radiographic results were documented based on the OFP grade.

2.4. Data analysis and statistical analysis

Clinical manifestations were compared between infants with OFP and infants without OFP, including sepsis, NEC, patent ductus arteriosus, cholestasis, number of births, and mode of delivery. The duration of the PN was analyzed with regard to the presence of OFP using receiver operating characteristic (ROC) curve analysis. The correlation between ALP levels and duration of PN was analyzed using Spearman's correlation. Quantitative data are described as means, medians, ranges, and standard deviations (SDs). Qualitative data are described using frequencies and percentages. Intergroup comparisons are described using independent *t* tests and chi-squared tests.

All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp, Armonk, NY); this included descriptive and inferential analyses (chi-squared, independent *t* tests, ROC curve analyses, and Spearman's correlations). A P value $< .05$ was considered significant.

3. Results

Of the 88 infants who met the inclusion criteria, 53 had incomplete dataset and could not, therefore, be analyzed. Consequently, only 35 infants were deemed eligible for inclusion. However, of the 35 infants, 2 were excluded because they did not survive until the end of the study, and 3 were withdrawn from the study. Therefore, a total of 30 infants were included in the final analysis. The clinical characteristics of these 30 infants are shown in Table 1. The BW of infants ranged from 800 g to 1495 g, and 5 (17%) had a BW <1000 g. The gestational age ranged from 27 to 32 weeks (mean ± SD, 29 ± 1.5 weeks). Details of individual patients are presented in Table 2.

PN was given for an average of 17 ± 10 (range, 4–50) days. The mean duration of receiving full enteral nutrition was 19 ± 8 (range, 7–37) days. The mean duration of hospitalization in all infants was 36 ± 10 (range, 14–60) days, whereas the mean duration of hospitalization in infants with OFP was 39 ± 11 (range, 23–60) days. Radiographs of the wrists showed signs of osteopenia in 13 infants (43%). The remaining 17 patients (57%) had normal wrists. No significant differences were found between infants with and without OFP with regard to the clinical characteristics, except for a higher incidence of NEC in the OFP group (Tables 1 and 2). Eleven infants were classified as having OFP grade 1 and 2 infants as OFP grade 2. Moreover, none of the infants showed symptoms associated with OFP grade 3, such as fractures.

Table 1

Characteristics of infants with and without osteopenia of prematurity (n = 30).

Variable	OFP (n = 13) n	No OFP (n = 17) n	*P* value‡
Sex			
Male	7	10	.785
Female	6	7	
Birth weight (g)			
1250 to 1500	6	10	.673
1000 to <1250	5	4	
750 to <1000	2	3	
Gestational age (wk)			
27	0	2	.334
28	6	2	
29	1	1	
30	4	7	
31	1	2	
32	1	3	
Sepsis	6	3	.091
Necrotizing enterocolitis	5	1	.027*
Patent ductus arteriosus	4	1	.070
Cholestasis	0	0	N/A
Multiple birth	0	3	.110
Mode of delivery			
Spontaneous	10	14	.713
Caesarean section	3	3	

N/A = not available, OFP = osteopenia of prematurity.
* *P* values <.05 were considered statistically significant.
‡ Chi-squared test.

The duration of PN was 8.8 days longer (95% confidence interval, 1.6–16.0) in infants with OFP than in infants without OFP ($P = .018$). The median duration of PN in the OFP group was 16 (range, 6–50) days, whereas the median duration of PN in the group without OFP was 12 (range, 4–21) days (Fig. 1). For each day of PN, the risk of OFP increased, with an odds ratio of 1.12 (95% confidence interval, 1.002–1.25; $P = .045$).

ALP levels increased with increasing postnatal age, from 208 ± 89 U/L before starting PN to 294 ± 166 U/L at the end of PN and to 328 ± 197 U/L before discharge. ALP levels around term age were higher in the OFP group than in the group without OFP (429 ± 260 vs 252 ± 72; $P = .01$). The duration of PN also correlated positively with ALP 2 (rho = 0.406; $P = .026$) and ALP 3 (rho = 0.488; $P = .006$) (Fig. 2). As expected, no correlation was detected between the duration of PN and ALP 1.

To further evaluate the relationship between the duration of PN and incidence of OFP, we constructed an ROC curve (Fig. 3). The area under the curve (AUC) was 0.667 (95% confidence interval, 0.455–0.880) with a P value of .121. Using the ROC curve, it was evident that the duration of PN with a 15-day cutoff had a sensitivity of 69.2% and a specificity of 70.6% for detecting the existence of OFP. Of the 30 infants enrolled in this study, 16 (53.3%) received PN for 15 days or <15 days, whereas 14 (46.7%) received PN for more than 15 days. PN for more than 15 days increased the risk of OFP, with an odds ratio of 5.40 (95% confidence interval, 1.12–26.04; $P = .035$).

Table 2

Clinical data of each infant (n = 30).

Sample	Birth weight (g)	ALP 1 (U/L)	ALP 2 (U/L)	ALP 3 (U/L)	Wrist radiograph	Parenteral nutrition duration (d)
Sample#1	1400	158	188	178	Normal	5
Sample#2	1120	275	270	314	Normal	20
Sample#3	1300	261	171	223	Normal	16
Sample#4	1400	55	276	263	OFP grade 1	8
Sample#5	1400	247	315	347	Normal	10
Sample#6	1490	327	417	379	OFP grade 1	29
Sample#7	1000	249	356	335	OFP grade 1	50
Sample#8	1100	228	345	249	Normal	18
Sample#9	900	162	364	362	OFP grade 1	16
Sample#10	1300	156	253	298	Normal	21
Sample#11	1300	183	581	315	Normal	21
Sample#12	1100	158	249	237	OFP grade 1	10
Sample#13	1400	218	364	513	OFP grade 1	16
Sample#14	1400	388	967	1203	OFP grade 2	33
Sample#15	1400	206	227	209	Normal	4
Sample#16	850	280	171	176	Normal	12
Sample#17	1050	230	300	578	OFP grade 2	40
Sample#18	1450	180	212	247	OFP grade 1	6
Sample#19	1400	410	505	538	OFP grade 1	18
Sample#20	1485	213	282	400	OFP grade 1	30
Sample#21	1490	141	183	221	OFP grade 1	16
Sample#22	800	142	158	170	Normal	10
Sample#23	1495	200	240	379	Normal	10
Sample#24	1000	152	258	399	OFP grade 1	30
Sample#25	1200	128	157	200	Normal	13
Sample#26	1300	115	150	180	Normal	10
Sample#27	1400	196	200	230	Normal	12
Sample#28	800	90	190	266	Normal	14
Sample#29	1400	100	125	135	Normal	11
Sample#30	1200	85	110	286	Normal	11

No cases were classified as OFP grade 3. The duration of parenteral nutrition refers to the time interval from the start of parenteral nutrition until the infants reached full enteral nutrition (150 mL/kg/d). ALP 1 (U/L) = serum ALP activity (U/L) at the start of parenteral nutrition, ALP 2 (U/L) = serum ALP activity (U/L) at the end of parenteral nutrition, ALP 3 (U/L) = serum ALP activity (U/L) at discharge, OFP = osteopenia of prematurity.

4. Discussion

In this study, we found that 43% of preterm infants with a BW <1500 g and cared for in the NICU had OFP around term age. The incidence of OFP was strongly associated with the duration of PN.

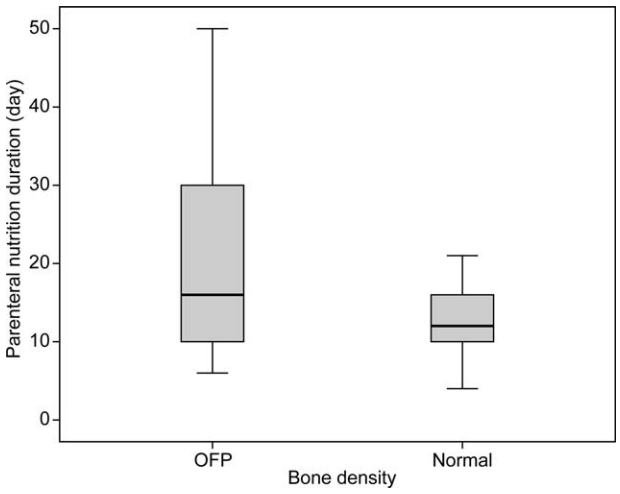
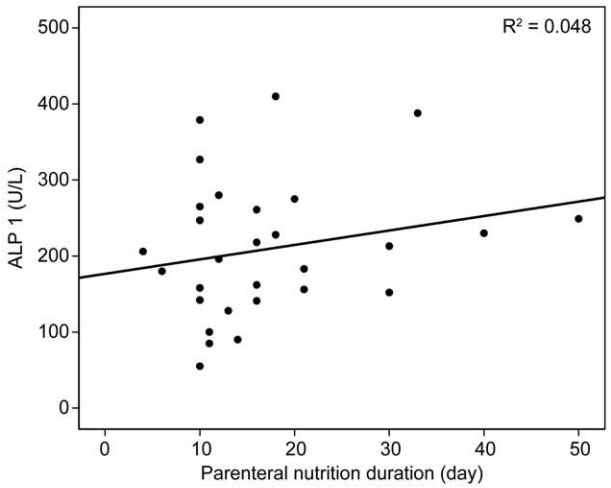
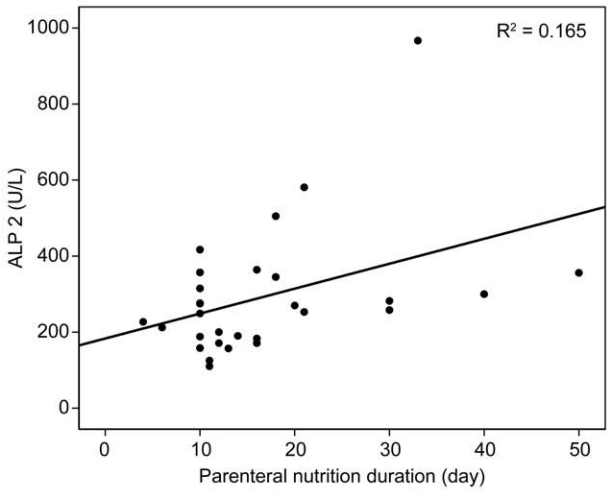


Figure 1. Median (range) of the duration of parenteral nutrition (days) in infants with OFP and infants without OFP (normal bone density). An independent *t* test showed a mean difference of 8.8 (95% confidence interval, 1.6–16; $P = .018$), thus indicating a significant difference between the 2 groups. Data are shown in the form of a box and whisker plot. OFP = osteopenia of prematurity.

OFP occurs in preterm infants when there is inadequate bone mineralization. The clinical onset of OFP is usually 6 to 12 weeks postnatally.[21] In a normal pregnancy, 80% of the stores of calcium, phosphate, and magnesium are built up during the last trimester.[22,23] Thus, the lower the gestational age of the infant, the lower the bone mineral reserves, consequently increasing the risk of OFP.[1,2] Besides prematurity, several other risk factors include bronchopulmonary dysplasia, duration of PN, corticosteroid, furosemide, methylxanthines, and a low intake of calcium, phosphate, and vitamin D.[23,24] Over recent times, the immobility of preterm infants after birth has also been considered to play a role in the incidence of OFP.[25] We excluded infants receiving long-term corticosteroids, furosemide, and/or methylxanthine therapy. Of the 13 infants with OFP, 11 had a BW ≥1000 g. Extreme prematurity therefore might not be the most likely explanation for our cases of OFP. In this study, we failed to find any significant differences between the groups with and without OFP in terms of clinical variables, except for a higher incidence of NEC in the OFP group. Previously, Cakir et al[26] reported a positive correlation between NEC and increased bone resorption in premature infants. This might be related to the reduced levels of glucagon-like peptide-2, an intestinal hormone that is predominantly secreted from the distal small intestine. The primary stimulus of glucagon-like peptide-2 secretion is enteral nutrient intake. Prolonged PN decreased enteral nutrient intake and contributed to the reduced levels of glucagon-like peptide-2 secretion.[26]

However, we did find a clear difference in the duration of PN between the 2 groups. The group without OFP received PN for 12 days on average, compared with 16 days in the OFP group. A





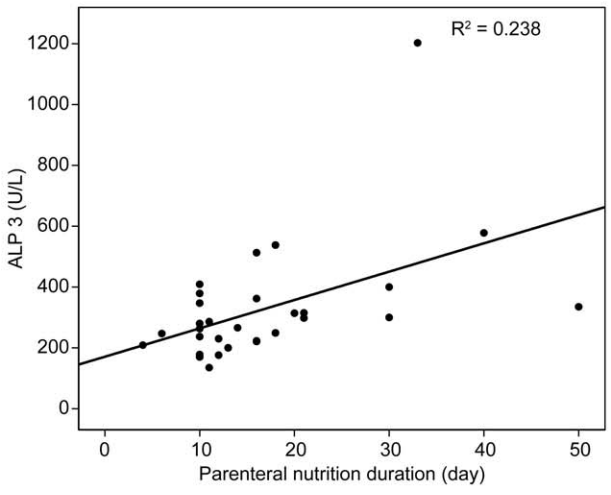


Figure 2. Scatter diagram showing ALP levels (U/L) with parenteral nutrition duration (days). The duration of parenteral nutrition was positively and significantly correlated with ALP 2 (rho = 0.406; $P = .026$) and ALP 3 (rho = 0.488; $P = .006$). No correlation was found between the duration of parenteral nutrition and ALP 1 (rho = 0.220; $P = .243$). ALP 1, serum ALP activity (U/L) at the start of parenteral nutrition; ALP 2, serum ALP activity (U/L) at the end of parenteral nutrition; ALP 3, serum ALP activity (U/L) at discharge.

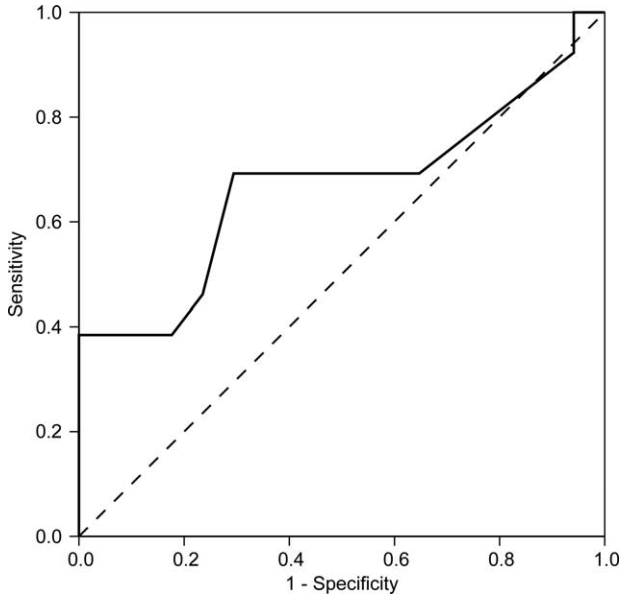


Figure 3. Receiver operating characteristics curve showing the duration of parenteral nutrition for detecting the existence of osteopenia of prematurity. The area under the curve was 0.667 (95% confidence interval, 0.455–0.880; $P = .121$). The duration of parenteral nutrition with a 15-day cutoff had a sensitivity of 69.2% and a specificity of 70.6% for the detection of osteopenia of prematurity.

number of studies have also found a moderate correlation between the duration of PN and development of OFP.[4,11] Based on the ROC curve, we divided our patients into 2 groups: a longer PN group (receiving PN >15 days) and a shorter PN group (receiving PN ≤ 15 days). The incidence of OFP was significantly higher in the longer PN group than in the shorter PN group. Several other studies have also reported that the incidence of OFP was higher in infants receiving PN for >14 days.[4,11] However, no previous study has investigated why a longer duration of PN is related to the development of OFP. The most likely explanation is the lack of an adequate intake of minerals and/or vitamin D. The accretion of calcium during the last trimester of normal pregnancy is 140 mg/kg/d (3.5 mmol/kg/d), whereas that for phosphate is 75 mg/kg/d (2.4 mmol/kg/d).[22] Adding these amounts of calcium and phosphate to the PN solution is difficult. When administering PN for a longer period, following our protocol, we provided 60 to 90 mg/kg/d (0.6–1.5 mmol/kg/d) of calcium and 40 to 160 IU/kg/d of vitamin D. However, we did not provide phosphate with the PN, as phosphate solutions for intravenous use are not available in Indonesia. We hypothesize that the high incidence of OFP, also seen in preterm infants with a BW >1000 g, is related to a lack of phosphate intake during PN. The unfortified preterm human milk contains 20 mg/100 mL of calcium and 15 mg/100 mL of phosphorus. This amount cannot fully meet the needs of preterm infants for calcium and phosphorus, even if the absorption of calcium and phosphorus is as much as 80% of dietary intake. Human milk fortification can increase calcium content up to 140 mg/100 mL and phosphorus content up to 80 mg/100 mL; therefore, human milk fortification is recommended for preterm infants to be able to meet the needs of bone mineralization. Fortification of preterm human milk increases bone mineralization in very low birth weight infants represented by higher bone mineral content values

and lower ALP values.[12,13] The practice of not using fortified human milk also contributes to the incidence of OFP. In an effort to optimize the nutritional support of human milk even without fortification, we provided oral vitamin D supplementation of 400 IU per day to meet the needs of bone mineralization in premature infants.[22,27]

The gold standard for measurement of bone density is dual-energy X-ray absorptiometry. This technique is not currently available in our hospital. Therefore, we used wrist radiography to detect OFP. We found that the ALP, which was carried out at 5 to 6 weeks after birth (or around term age), was significantly higher in the OFP group than in the group without OFP. You et al[28] demonstrated that taking wrist radiography supported by ALP examination measured at around 6.9 weeks after birth was reliable for diagnosing OFP. Based on the radiograph results, we found 11 infants classified as having OFP grade 1, 2 infants classified as having OFP grade 2, and no infants showing signs of fracture or OFP grade 3. Other studies that used radiography of the wrist to establish a diagnosis of OFP have reported that the incidence of OFP was 13.3%,[29] 39%,[30] and 39%[31] in infants with a BW <1500 g. These results are lower than those observed in our study. The reduction in bone mineralization could only be seen on radiographs when bone mineralization had decreased by at least 20% to 40%. Radiographic abnormalities are therefore rather late signs of OFP.[1] Radiograph assessment to detect the presence of OFP in our study was carried out by an experienced radiologist. O'Reilly et al[30] showed that radiograph assessment by an experienced radiologist significantly improved the detection ability of OFP, even though radiography alone is not sufficient to assess the presence of OFP. These findings prove the importance of screening for early detection and prevention of OFP in preterm infants while continuing to optimize adequate nutrition. Quantitative ultrasonography at the tibia level is also a useful modality for investigating the presence of OFP. Although it can detect bone density more accurately, it is difficult to use as a routine procedure; hence, it has not been widely used in clinical practice.[1,28]

Previous research has demonstrated that serum ALP is a reliable marker of bone turnover and that high ALP levels reflect increased levels of cellular activity in bone. ALP has been used in a number of previous studies as an indicator of OFP.[29,31] Serial ALP measurements have also been reported to improve the diagnostic accuracy of OFP.[29,31] A previous study found that ALP levels of more than 1000 U/L provide significant support for the diagnosis of OFP.[32] Recent studies have shown that ALP levels were lower than in previous studies to support the diagnosis of OFP. Hung et al[31] reported ALP levels of >700 U/L to predict OFP at term age with a sensitivity of 73% and specificity of 73%. Abdallah et al[29] and Figueras-Aloy et al[33] reported lower ALP levels in the diagnosis of OFP (>500 U/L) and suggested that ALP levels indicated a mild OFP. However, the cutoff ALP level for OFP has not been established to date.[2,33] In our study, only 4 of the OFP infants had an ALP level of >500 U/L. We observed lower ALP levels when diagnosing OFP than in other studies; however, we measured ALP in a serial manner. These findings are consistent with those reported by Abdallah et al[29]; however, the factors underlying such results remain unclear. One explanation is that in the absence of liver disorders, the cut-off level for serum ALP as a marker of bone disorders (500 U/L) is >4 times the level of serum ALP in adults. Nevertheless, we found that ALP levels were significantly higher in infants with abnormal wrist examination results than in those with normal wrist examination results, thus supporting the fact that we had classified infants with OFP correctly. Almost all ALP levels in both groups were within the so-called reference range.[34] At the same time, we found an increase in ALP levels from the first measurement at the beginning of PN to the levels measured at term age. ALP levels were positively correlated with the duration of PN and were higher in the OFP group than in the control group at term age. We suggest that an increase in ALP level might indicate the development of OFP, but it is not a diagnostic marker.

This study had a few limitations that need to be considered. First, our sample size was relatively small. However, in this small group, we found that nearly 43% of preterm infants showed signs of OFP. We suggest that a larger study, using more markers for OFP than we were able to obtain in our setting, is needed to further define the incidence of OFP in preterm infants in a developing country and to identify the causes. Second, we did not use parathyroid hormone (PTH), phosphate, urinary calcium and phosphate excretion, and rate of phosphorus reabsorption as markers of OFP. The homeostasis of calcium and phosphorus is regulated by PTH. OFP can be caused by a deficiency of calcium and phosphate. In case of a calcium deficiency, an increase in PTH is observed, but this is not found in case of a phosphate deficiency. Urinary biomarkers might vary considerably from day to day, or even within a day, and are highly dependent on the dietary intake. Calcium examination cannot be used as a reliable marker for OFP because calcium results can be normal even though bone calcium loss has occurred.[1,2,32] In this study, we did not conduct a PTH examination in our patients. Nonetheless, we hypothesized that the OFP in our patients was due to a phosphate deficiency. We added calcium to the PN and monitored the serum calcium levels to ensure that it stayed within the normal range. No phosphate was supplemented to the PN. Whether the ALP levels are lower in case of phosphate than in calcium deficiency is not known, this needs to be confirmed in a further study. However, we found radiographic abnormalities and increased ALP results, both of which can adequately reflect OFP in these preterm infants. The OFP remains a significant problem in preterm infants which may have an influence on long-term consequences.[6] Further research should be carried out on the prevention of OFP in a multicenter setting, including facilities with limited availability for the nutritional support (PN and enteral nutrition) of preterm infants. Moreover, longitudinal studies are needed to evaluate OFP over the long term in such conditions.

5. Conclusion

We found OFP in 43% of preterm infants with a BW between 800 and 1495 g admitted to our NICU. An association was found between the duration of PN and incidence of OFP. We hypothesize that the high incidence of OFP as well as the association with the duration of PN is caused by a lack of phosphate in the PN. Studies on the incidence of OFP in other developing countries with limited access to phosphate solutions are warranted. Moreover, further research is now needed to develop optimal methods for the prevention of OFP.

Acknowledgments

The authors gratefully acknowledge Hari Basuki Notobroto, MD, for his assistance with the statistical analysis and all the doctors in the Neonatology Division at the Dr. Soetomo General

Academic Hospital and the Post-Graduate Doctoral Program Faculty of Medicine, Airlangga University, Surabaya, Indonesia, for supporting this study. The authors also acknowledge Firas Farissy Alkaff and Kinanti Ayu Ratnasari for providing writing support. They would also like to thank Editage (www.editage.com) for English language editing.

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