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Associated neonatal and maternal factors of osteopenia of prematurity in low resource setting: A cross-sectional study

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ABSTRACT

Objective: Determining neonatal and maternal factors that are associated with the incidence of OFP.

Methods: This study employed a cross-sectional design, in which the participants were identified for clinical variables (sex, gestational age, birth weight, etc.), neonatal morbidity (sepsis, necrotizing enterocolitis (NEC), etc.), and maternal risk factors (premature rupture of membranes, preeclampsia, etc.). The data were analyzed using Chi-square test, independent t-test, and logistic regression test with $p < 0.05$.

Results: The birth weight ranged from 800 to 1495 g (1219 ± 225 g), of which 5 newborns (17%) were <1000 g. The gestational age ranged from 27 to 32 weeks, with a mean of 29 ± 1.5 weeks. The signs of OFP were observed in 13 (43%) infants, of which 2 (15%) OFP infants had a birth weight <1000 g. There was significant difference in parenteral nutrition duration ($p = 0.018$), onset of vitamin D supplementation ($p = 0.019$), and ALP level ($p = 0.012$) of infants between the OFP group and the non-OFP group. The variables associated with the incidence of OFP were parenteral nutrition duration >15 days (OR = 5.4; 95% CI 1.120–26.044; $p = 0.036$), ALP level >500 U/L (OR = 2.889; 95% CI 1.703–4.900; $p = 0.014$), and PROM (OR = 5.4; 95% CI 1.039–28.533; $p = 0.045$).

Conclusion: The lack of phosphate intake, prolonged parenteral nutrition, ALP level >500 U/L, onset of vitamin D supplementation, and premature rupture of membranes are associated with the incidence of OFP.

1. Introduction

Osteopenia of prematurity (OFP) is characterized by a decrease in bone mineral content (calcium and phosphorus) that mostly occurs in preterm infants, as a consequence of transplacental interruption of mineral transfer which mainly occurs in the third trimester [1]. The incidence of OFP is highly correlated with birth weight and gestational age. Previous studies reported that OFP occurred in 10% of low birth-weight infants, 20% of those weighing <1500 g, and 30–60% of infants weighing <1000 g [2]. Furthermore, the disease possibly causes fractures [3], poor respiratory outcome [4], inadequate weight gain [5], impaired growth [6], and predisposing risk of osteoporosis in adulthood [7].

Despite advancement of technology in preterm infants' care, the incidence of OFP remains to exist [8]. A decrease in bone mineralization can be seen through radiograph when there is a 40% reduction in bone; however, it makes the diagnosis of OFP a challenge [1]. Research

evidence demonstrated that, apart from prematurity and birth weight, there were other risk factors detected that might increase the risk of OFP [9]. Placental insufficiency, as occurred in pre-eclampsia and chorioamnionitis, impaired mineral transfer in utero [10]. Inadequate calcium and phosphorus supplementation, late supplementation of vitamin D, late achievement of total enteral nutrition, and prolonged use of parenteral nutrition have been identified the risk factors for OFP [11]. Necrotizing enterocolitis (NEC) [12], sepsis [13], cholestasis [9], lacking of mobilization [14], and using specific drugs, such as furosemide, methylxanthine, and corticosteroid, also contributed to the incidence of OFP [11]. The etiology of OFP therefore represents a multifactorial and complex origin; however, the primary pathogenesis of OFP leads to deficiency of mineral (calcium and phosphorus) and/or vitamin D levels [8,9]. Identification of risk factors helps provide specific therapeutic interventions for preterm infants with OFP in order to promote better outcome [9].

The rate of preterm birth in Indonesia is 15 per 100 live births.

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Indonesia ranks fifth in the world in the number of preterm births. The high number of preterm infants suggests that Indonesia, as a developing country, needs a strategy to improve survival and outcome in preterm infants including the prevention of the occurrence of OFP [15]. However, the OFP must be detected early so that appropriate management strategies can be put into place to avoid serious long-term complications [2]. To our knowledge, this is the first study in Indonesia that investigated both infant and maternal factors associated with the incidence of OFP. Thus, the aim of this study was to determine both neonatal and maternal factors that were associated with the incidence of OFP.

2. Method

2.1. Participant

Preterm infants with a birth weight <1500 g who were admitted to Hospital were taken into consideration as the study participants. Infants with multiple congenital abnormalities and congenital heart defects with fluid restriction were excluded in this study. This study also

excluded infants who ceased before bone mineralization could be measured, the infants of parents who did not provide consent, and infants who were withdrawn from the study by their parents. Of the 98 infants who met the inclusion criteria, 53 had incomplete dataset, 5 did not have parental consent, 5 had multiple congenital anomaly, 2 did not survive until the end of the study, and 3 were withdrawn from the study for personal reasons. Therefore, a total of 30 infants were included in the final analysis. The process of participant's recruitment was described in Fig. 1. The 30 participants are divided into 2 groups, namely OFP group (13 participant) and non-OFP group (17 participant).

2.2. Ethical approval

This study was approved base on Declaration of Helsinki by the Ethical Committee of Health Research at Hospital, Surabaya. The data were collected from each subject following the approval acquisition by either parents or other representatives in form of written informed consents.

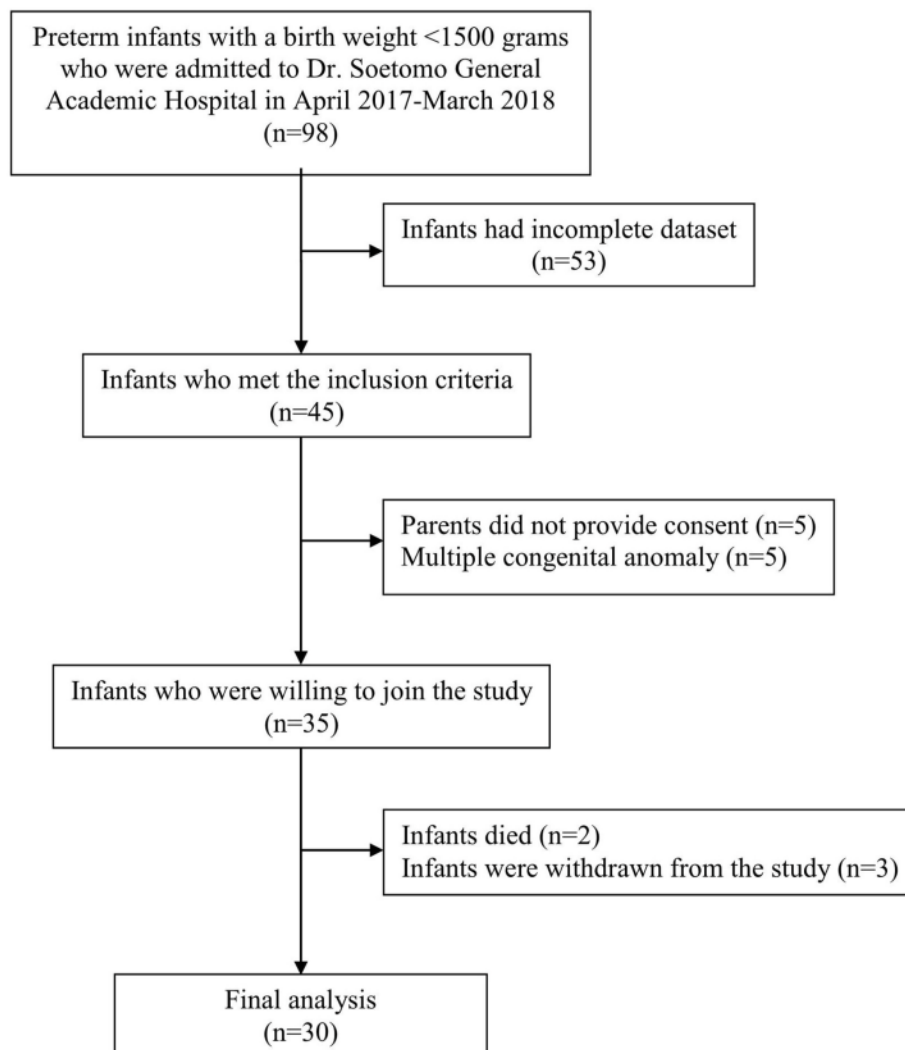


Fig. 1. The process of participant's recruitment.

2.3. Study design

This study used a cross-sectional design that was conducted in neonatal intensive care unit (NICU) of Hospital, Surabaya, Indonesia. The study was carried out from April 2017 to March 2018 in which this reporting uses the STROCSS 2019 Guideline [16]. The researchers collected a range of clinical variables, including sex, gestational age, birth weight, multiple birth, mode of delivery, laboratory findings (serum calcium and serum ALP), onset of vitamin D supplementation, duration of parenteral nutrition, duration of full enteral feeding, and length of hospitalization. Moreover, this study collected neonatal morbidities such as sepsis, necrotizing enterocolitis (NEC), patent ductus arteriosus (PDA), and cholestasis. Apart from infant data, there were maternal factors collected, such as premature rupture of membranes (PROM), pre-eclampsia, and maternal age >35 years old.

2.4. Nutritional protocol

All infants received parenteral nutrition according to the standard protocol in the NICU, beginning on the first day after birth. Intravenous amino acids (Aminosteril Infant; Fresenius Kabi, Bad Homburg, Germany) were given with a starting dose of 2 g/kg/day and increased daily by 0.5 g/kg/day to 3.5 g/kg/day. The researchers provided glucose supply with a glucose infusion rate at least 4 mg/kg/min and maximum 12 mg/kg/min. Intravenous lipids (Smoflipid 20%; Fresenius Kabi, Bad Homburg, Germany) were administered with a starting dose of 1 g/kg/day and increased daily by 0.5 g/kg/day to 3 g/kg/day [17]. Several electrolytes were provided including sodium (2–4 mmol/kg/day), potassium (1–2 mmol/kg/day), magnesium (0.1–0.3 mmol/kg/day), and calcium (0.6–1.5 mmol/kg/day). No phosphate solution was added. 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/day and 4 mL/kg/day, respectively. Serum electrolyte tests (sodium, potassium, calcium, and chloride) were carried out every week or as indicated [18]. Vitamin D was provided at a dose of 400 IU/day orally when enteral feeding achieved 100 mL/kg/day [19]. The total fluid volume was started at 80 mL/kg/day, then increased daily by 10–20 mL/kg/day until the target volume of 180 mL/kg/day was achieved.

Enteral feeding was administered on the first day of life at a dose of 10 mL/kg/day. If the infant tolerated this amount, the volume was increased by 20 mL/kg/day. The infants received breast milk or preterm formula milk should breast milk was not available. Parenteral nutrition was stopped when the enteral feeding volume had achieved 120 mL/kg/day; then the infants received full enteral feeding until discharged.

2.5. Diagnosis of OFP

The diagnosis of OFP was confirmed using the X-ray findings of both wrists, which was performed by a certified radiologist and further classified in accordance with the scoring system of Koo [20]. Normal bone density was characterized by a normal dense white line that presented at the metaphysis with a normal band of lucency in the sub-metaphyseal region. OFP was characterized by the disappearance of normal dense white line at the metaphysis with increased sub-metaphyseal lucency and thinning of the cortex, irregularity and fraying of the metaphysis, cupping, or evidence of fractures. In the basis of OFP in wrist radiography, this study classified infants into the OFP groups and the non-OFP groups. Serum alkaline phosphatase (ALP) was measured at the third week, then the ALP examination was repeated every week. Wrist radiography was performed at the time the infant was discharged, or when ALP level >500 U/L, or there were clinical symptoms of OFP such as fractures. This study recorded serum ALP and serum calcium levels when the OFP diagnosis was established.

2.6. Data analysis and statistical analysis

Quantitative data were described using mean, range, and standard deviation (SD). Qualitative data were described using frequencies and percentages. Intergroup comparisons were analyzed using Chi-square test (for categorical variables) and *t*-test (for continuous variables). The odds ratio (OR) was conducted using logistic regression analysis and Chi-square test. Statistical significance was set at *P* value of ≤0.05 and 95% confidence interval (CI). All statistical analyses were performed using IBM SPSS Statistics 21 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Characteristics of participants

Of the total 30 infants, the birth weight ranged from 800 to 1495 g (1219 ± 225 g), of which 5 infants (17%) were <1000 g. The gestational age ranged from 27 to 32 weeks, with a mean of 29 ± 1.5 weeks. Based on the X-ray findings of the wrists, the signs of OFP were observed in 13 (43%) infants, of which 2 (15%) in OFP infants had a birthweight <1000 g. There was no significant difference in sex, birth weight, gestational age, multiple birth, mode of delivery, serum calcium, full enteral feeding duration, and length of hospitalization of infants between the OFP group and the non-OFP group. However, there was significant difference in parenteral nutrition duration (*P* = 0.018), onset of vitamin D supplementation (*P* = 0.019), and ALP level (*P* = 0.012) between infants in the OFP group and the non-OFP group. The detail of characteristics of all infants based on the presence of OFP were presented in Tables 1 and 2.

3.2. Determinant factor on osteopenia of prematurity in Indonesian infant

Seven variables of neonatal factors were analyzed for development of OFP, including parenteral nutrition duration >15 days, onset of vitamin D supplementation at >14 days of age, sepsis, NEC, PDA, cholestasis, and ALP level >500 U/L (Table 3). The variables that increased the risk of OFP were parenteral nutrition duration >15 days (OR = 5.4, 95% CI = 1.120–26.044; *P* = 0.036) and ALP level >500 U/L (OR = 2.889, 95% CI = 1.703–4.900; *P* = 0.014).

Three variables of characteristic of mothers were analyzed for development of OFP, including PROM, pre-eclampsia, and maternal age >35 years old (Table 4). The variable that increased the risk of OFP was PROM (OR = 5.4, 95% CI = 1.039–28.533; *P* = 0.045).

Table 1
Characteristics of all infants based on the presence of OFP.

Variable	OFP group n = 13 (%)	Non-OFP group n = 17 (%)	<i>p</i>
Sex			0.785
Male	7 (54)	10 (59)	
Female	6 (46)	7 (41)	
Birth weight (gram)			0.673
1250–1500	6 (46)	10 (59)	
1000 – <1250	5 (39)	4 (23)	
<750 – <1000	2 (15)	3 (18)	
Gestational Age (weeks)			0.334
27	0 (0)	2 (12)	
28	6 (46)	2 (12)	
29	1 (8)	1 (6)	
30	4 (30)	7 (41)	
31	1 (8)	2 (12)	
32	1 (8)	3 (18)	
Multiple birth	0 (0)	3 (18)	0.110
Mode of delivery			0.713
Spontaneous	10 (77)	14 (82)	
Caesarean section	3 (23)	3 (18)	

Table 2
Characteristics of participant based on distribution frequency.

Variable	OFF group n = 13 (Mean ± SD)	Non-OFF group n = 17 (Mean ± SD)	p
Onset of vitamin D supplementation	19 ± 13.4	10 ± 4.7	0.019*
Parenteral nutrition duration (day)	22 ± 13	13 ± 4	0.018*
Full enteral feeding duration (day)	17 ± 7	21 ± 8	0.152
Length of hospitalization (day)	39 ± 11	34 ± 9	0.262
Serum calcium (mg/dL)	8.4 ± 0.31	8.3 ± 0.23	0.786
Serum alkaline phosphatase (U/L)	429 ± 260	252 ± 72	0.012*

Note: *Significance $p < 0.05$.

Table 3
Univariate analysis in osteopenia of prematurity.

Variable	Group		OR	95% CI	p
	OFT n = 13 (%)	Non-OFT n = 17 (%)			
Parenteral nutrition duration >15 days	9 (69.2)	5 (29.4)	5.400	1.120–26.044	0.036*
Onset of vitamin D supplementation at >14 days of age	6 (46.2)	4 (23.5)	2.786	0.583–13.305	0.199
Sepsis	6 (46.2)	3 (17.6)	4.000	0.763–20.963	0.101
Necrotizing Enterocolitis	5 (38.5)	1 (5.9)	10.000	0.994–100.612	0.051
Patent Ductus Arteriosus	4 (30.8)	1 (5.9)	7.111	0.686–73.714	0.100
Cholestasis	0	0	0.765	N/A	0.467
ALP level >500 U/L	4 (30.8)	0	2.889	1.703–4.900	0.014*

Note: OR, odds ratio; CI, confidence interval; SD, standard deviation; OFF, osteopenia of prematurity; *Significant $p < 0.05$; N/A, not available.

Table 4
Characteristics of mothers in osteopenia of prematurity.

Variable	Group		OR	95% CI	p
	OFT n = 13 (%)	Non-OFT n = 17 (%)			
Premature rupture of membrane	7 (53.8)	3 (17.6)	5.444	1.039–28.533	0.045*
Pre-eclampsia	6 (46.2)	11 (64.7)	0.468	0.107–2.046	0.313
Maternal age >35 years old	4 (30.8)	8 (47.1)	0.500	0.110–2.274	0.370

Note: OR, odds ratio; CI, confidence interval; SD, standard deviation; OFF, osteopenia of prematurity; *Significant $p < 0.05$.

4. Discussion

OFF, that is frequently observed in preterm infants, is characterized by the lack of mineralization in bones which on a severe clinical feature may indicate fracture even under normal handling [2]. The clinical sign of OFF usually appears between 5 and 11 weeks of postnatal age and may not even be visible until signs of fracture. Since the long term consequences of OFF have a negative impact on the growth and development of preterm infants, it makes prevention of OFF an important role [1]. With recent advances in the care of preterm infants, there has been improved survival of preterm infants; however, the morbidity of OFF was observed [21].

This study identified the incidence of OFF occurred in 43% of preterm infants with birthweight below 1500 g. Other studies reported the incidence of OFF that varied in the range from 10% [11] to 50% [9]. The varying incidence of OFF appeared to be reliant on either the birth weight or gestational age of the infant, which indicated the OFF occurrence mostly occurs in extremely low birth weight infants. The incidence of OFF in this study was higher than that of other studies associated with the majority of preterm infants with OFF in this study (85%) having a birth weight of more than 1000 g.

The research evidence indicated that the etiology of OFF is multifactorial and complex in origin; however, the primary pathogenesis of OFF leads to inadequacy of bone mineral requirements as a consequence of transplacental interruption of mineral transfer in preterm birth [1, 21]. Postnatally, if the intake of calcium and phosphorus of preterm infants cannot meet their needs, there will be a lack of mineralization in the bones that can lead to the occurrence of OFF [22]. The calcium accretion rate during last trimester intrauterine was 140 mg/kg/day (3.5 mmol/kg/day), while that for phosphate was 75 mg/kg/day (2.4 mmol/kg/day). It is difficult to meet the needs of these minerals, such as intrauterine both enteral nutrition and parenteral nutrition, because of the limited amount that can be given and limited intestinal absorption [22]. This study's parenteral nutrition protocol provided 60–90 mg/kg/day (0.6–1.5 mmol/kg/day) of calcium and 40–160 IU/kg/day of vitamin D. Phosphate could not be administered alongside standardized PN, as phosphate solutions for intravenous use are currently not ubiquitous in Indonesia.

This study found a significant difference of parenteral nutrition duration between OFF group and non-OFF group; therefore, parenteral nutrition duration >15 days was a significant risk factor for increasing the incidence of OFF. Previous study reported that the longer the administration of parenteral nutrition increased the risk of OFF due to the contamination of the aluminum which caused impaired bone metabolism [9,23]. The aluminum is released from the glass vial into the solutions during the sterilization process, which is quite arduous to distill. For this reason, prevention method may come imperative by having the transition from parenteral to enteral feeding initiated earlier [9]. Moreover, neonatal morbidity such as NEC has an impact on the longer duration of parenteral nutrition, of which there is evidence of a positive correlation between NEC and increased bone resorption [12]. Therefore, this study hypothesized that a lack of phosphate intake in parenteral nutrition as well as prolonged parenteral nutrition contributed to the high incidence of OFF. For calcium, this study added calcium solution to parenteral nutrition and maintained serum calcium level within normal ranges.

Full enteral feeding duration had no correlation with the incidence of OFF. These findings were put down to varying approaches of enteral feeding given to infants, likewise breast milk, fortified breast milk, or preterm formula; however, breast milk fortification is not carried out routinely in the NICU the study took place. The practice of not administering fortified breast milk is held accountable for OFF occurrence as the unfortified preterm breast milk contains 20 mg/100 mL of calcium and 15 mg/100 mL of phosphorus. This amount, however, seems not commensurate with the preterm infants' requirements of calcium and phosphorus, even if the absorption of calcium and phosphorus constitute 80% of dietary intake. Breast milk fortification can increase calcium content up to 140 mg/100 mL and phosphorus content up to 80 mg/100 mL; hence, breast milk fortification is recommended for preterm infants to be able to meet the needs of bone mineralization [24]. Preterm formula provides calcium content to 100–150 mg/100 mL (50–60% absorption rate) and phosphorus content to 55–65 mg/100 mL (90% absorption rate) [25,26]. Calcium and phosphorus content in preterm formula is higher than standard formula, therefore, preterm formula is recommended to increase bone mineralization in preterm [27]. In effort to optimize the nutritional support of breast milk even without fortification, this study administered oral vitamin D supplementation of 400 IU on daily basis to attain the requirements of bone mineralization in

premature infants [28]. This study found that the onset of vitamin D supplementation in the OFP group was significantly more delayed than in the non OFP group. These findings are consistent with previous research conducted by Cho et al. which demonstrated that early vitamin D supplementation that initiated at 14 days after birth enhanced vitamin D status and improved bone mineralization in preterm infants [29].

This study found that PROM was identified as a significant factor that increased the incidence of OFP. Chorioamnionitis, which represented by PROM, is associated with placental insufficiency which results in impaired transplacental mineral transfer [10]. A prospective cohort study of 50 neonates with early onset sepsis reported that neonates born to PROM mothers had lower levels of 25-OH vitamin D compared with those neonates born to mothers without PROM [30]. However, vitamin D deficiency is highly associated with the incidence of OFP [31].

For laboratory findings, this study discovered that there was a significant increase in serum ALP level in the OFP group compared to non-OFP group, while calcium levels did not differ significantly between these two groups. These findings are consistent with those in other studies [32,33]. Abdallah et al. stated that serial ALP measurement was reliable to support the diagnosis of OFP, on contrary calcium examination is not yet ample for the diagnosis of OFP [32]. Calcium examination could not be used as a reliable marker for OFP because calcium results might remain normal despite calcium loss [34,35]. This study observed that the mean of serum ALP level in OFP group was 429 U/L, lower than those used in other studies. However, the ALP level >500 U/L is associated with increased risk of OFP. This finding is in accordance with a study conducted by O'Reilly et al. who revealed that rib fractures were apparently found in a preterm infant with the highest ALP level of 394 U/L; however, their infant had several risk factors such as bronchopulmonary dysplasia and NEC [3]. A study conducted by Figueras-Aloy et al. also showed that the ALP levels of 301–700 U/L in preterm infants indicated mild metabolic disease of prematurity [36]. The OFP can be caused by either phosphate deficiency or calcium deficiency. Whether ALP level is lower in case of phosphate deficiency than in a calcium deficiency is unclear, this needs to be investigated in further research [2].

This study had a few limitations that need to be considered. First, the sample size was rather small. However, in this small-sized group of samples, this study found that almost of 43% of preterm infants showed signs of OFP. Therefore, this study suggested that a larger study is still preferable to further explore the incidence of OFP in preterm infants, particularly in developing country, and to investigate the causes. However, there remain other factors may also likely relate to OFP risks, for instance certain drugs, such as corticosteroid, furosemide, or methylxanthines [37]. In this study, the data on these drugs were incomplete and therefore insufficient for analysis. Secondly, this study used X-ray to confirm the diagnosis of OFP. The gold standard to measure bone density is dual-energy X-ray absorptiometry. Quantitative ultrasound at the tibia level is also a reliable modality for investigating the presence of OFP. Other studies mostly used X-rays to diagnose OFP [32,38], this is because dual-energy X-ray absorptiometry and quantitative ultrasound are not routinely used for clinical practice and they are usually used for research purposes [2]. However, dual-energy X-ray absorptiometry and quantitative ultrasound are currently not available in the hospital where this study took place.

5. Conclusion

This study found high incidence of OFP in preterm infants with birthweight below 1500 g. The lack of phosphate intake, prolonged parenteral nutrition, ALP level >500 U/L, onset of vitamin D supplementation, and premature rupture of membrane are associated with incidence of OFP. Moreover, a larger study remains entailed to further define the incidence of OFP in preterm infants in developing countries and to investigate the causes for helping to improve the prevention of OFP and to promote better outcome of preterm infants.

Guarantor

Risa Etika is the person in charge for the publication of our manuscript.

Consent for publication

Not applicable.

Availability of data and material

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

Sources of funding

This research did not receive any specific grants from any funding agency in the public, commercial, and not-for-profit sectors.

Ethical approval

This study was approved base on Declaration of Helsinki by the Ethical Committee of Health Research at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia.

Author contribution

Dina Angelika: Conceptualization, Methodology, Data curation, Resources, Writing-original draft. Risa Etika: Visualization, Supervision, Investigating, Writing-original draft. Pradhika: Software, Resources, Data curation, Validation. Martono: Visualization, Supervision, Investigating. Paulus Rahardjo: Software, Resources, Data curation, Supervision, Investigating. Ugrasena: Methodology, Formal analysis, Writing-review and Editing. All authors read and approved the final manuscript.

Trail registry number

1. Name of the registry: Ethical Committee of Health Research at Dr. Soetomo General Academic Hospital, Surabaya, Indonesia
2. Unique Identifying number or registration ID: 370/Panke.KKE/V/192017
3. Hyperlink to your specific registration (must be publicly accessible and will be checked):

Guarantor

Risa Etika is the person in charge for the publication of our manuscript.

Declaration of competing interest

The authors declare that there was no conflicting interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.amsu.2021.102235>.

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