Comparison of daily 400 and 600 units of vitamin D in prevention of osteopenia of prematurity in infants with gestational age of less than and equal to 32 weeks

Behzad Barekatain (✉ b_barekatain@med.mui.ac.ir)
Isfahan University of Medical Sciences

Shima Hamidipour
Isfahan University of Medical Sciences

Zohreh Badiei
Isfahan University of Medical Sciences

Maryam Farghadani
Isfahan University of Medical Sciences

Research Article

Keywords: Osteopenia of prematurity, Calcium, Phosphorus, Alkaline phosphatase, Vitamin D

Posted Date: August 21st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3259219/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background

Use of vitamin D in premature infants is one of the important preventive factors for Osteopenia of prematurity but there are conflicting results on the appropriate doses, so this study aimed to compare the dose of 400 and 600 units vitamin D in the prevention of osteopenia of prematurity in infants with gestational age ≤ 32 weeks.

Methods

This clinical trial study was conducted on 108 premature infants divided into two groups of 54 with gestational age of ≤ 32 weeks born between 2020-2021 in Shahid Beheshti and Al-Zahrai hospitals in Isfahan. In the first group, daily vitamin D was 400 units from the 7th day of birth, and in the second group, it was 600 units. At the age of 5 weeks, levels of calcium, phosphorus, alkaline phosphatase and vitamin D were evaluated. If the alkaline phosphatase level was above 1000, the wrist radiography was requested.

Also, the baby was examined for clinical symptoms of rickets at the age of 5 weeks. The data were analyzed by SPSS software version 26 and P-value<0.05 was considered significant.

Results

In the present study, there was no significant difference between the level of alkaline phosphatase in the two groups (p=0.596), but the level of vitamin D was significantly higher in 600 units group (p<0.001). The level of calcium was higher in 400 units group, but this difference was slightly significant (p=0.062). The level of phosphorus in 600 units group was higher than the 400 units, and the difference was slightly significant (P=0.062).

Conclusion

The present study showed that daily 600 and 400 units of vitamin D in infants with gestational age of ≤ 32 weeks had no effect on the incidence of clinical symptoms and radiological findings of rickets at the age of 5 weeks.

Introduction

Preterm infants are exposed to an increased risk of bone disease, which is caused by a decline in the level and content of minerals in the body. This bone disease is known as osteopenia of prematurity or metabolic bone disease or rickets of prematurity and is more related to gestational age and birth weight. About 50% of infants with a birth weight of less than 1 kg and 23 to 32% of infants with a birth weight of less than 1.5 kg suffer from this disease (1). The peak incidence of this disease is 4 to 8 weeks after
birth. Most infants are asymptomatic at the time of diagnosis, and symptoms often appear 5 to 11 weeks after birth (2).

Some show non-specific symptoms, such as anorexia, vomiting, constipation, and rarely diarrhea. Infants are often lethargic, dehydrated, and hypotonic and may have seizures or FTT. Bone fracture and rickets are major symptoms of this disease (1). They may show an increase in respiratory work due to rib fractures and an increase in the size of brain sutures and frontal bossing (2).

Risk factors for metabolic bone disease include placental insufficiency, pre eclampsia, neuromuscular disorders, chorioamnionitis, Total Parenteral Nutrition (TPN) for more than 4 weeks, male sex, liver and kidney disease, necrotizing enterocolitis, periventricular leukomalacia, intraventricular hemorrhage, bronchopulmonary dysplasia, and the use of drugs such as loop diuretics, methylxanthines, and glucocorticoids (2). A simple x-ray of the wrist in infants with clinical symptoms of rickets and high alkaline phosphatase is a practical measure to evaluate bone changes (1). Biochemical tests to diagnose metabolic bone disease in premature infants include the evaluation of calcium, phosphorus, alkaline phosphatase, and vitamin D levels (3). In this disease, serum calcium concentration is usually normal and serum phosphorus concentration is normal or low. Also, the concentration of alkaline phosphatase increases during this disease (1). Hypophosphatemia is the first sign of destruction of mineral metabolism, occurs around 7 to 14 days after birth (2). A serum phosphorus level of less than 3.6 mg/dL (1.16 mmol/L) in breastfeed infants is considered as a risk factor for metabolic bone disease (2). A serum phosphorus level of less than 5.6 mg/dl (< 1.8 mmol/l) is strongly associated with the presence of radiological rickets in premature infants with the mean gestational age of 30.3 weeks and a mean birth weight of 1490 grams (4).

Alkaline phosphatase increases in the first three weeks of life and reaches to its peak in 6 to 12 weeks. Its level of more than 500 units/liter is associated with bone homeostasis destruction and the level of more than 700 units/liter with bone demineralization (2). Also, the level of more than 900 units/liter is a reliable indicator for osteopenia of prematurity, so the simultaneous measurement of phosphorus and alkaline phosphatase is a suitable method for screening (1). However, for screening, serum calcium level is not very reliable since it can be normal despite the loss of bone calcium (2). Hypocalcemia in infants refers to serum calcium level less than 8 mg/dl (2 mmol/l) (1).

Vitamin D is active in the regulation of calcium and phosphorus and supports the cellular process of bone mineralization and neuromuscular activity (4). Vitamin D deficiency is common in preterm infants as a result of problems in adequate enteral nutrition and lack of contact with sunlight that is occurred during hospitalization. According to the American Institute of Medicine, 20 ng/ml (50 nmol/l) of vitamin D and above is usually considered sufficient (1). Neonates often have little time to store maternal vitamin D, which is due to the decrease of vitamin D transferring through the placenta, so this problem leads to higher need of vitamin D because it is an essential element to activate calcium absorption (1). Also, infants who use only breast milk are more exposed to vitamin D deficiency.
In order to prevent osteopenia of prematurity in preterm infants, different doses of vitamin D is recommended in various sources, so this study aimed to compare the two different doses of vitamin D (400 units and 600 units) in preterm infants of less than and equal to 32 weeks.

**Materials and Methods**

This double-blind clinical trial was conducted in Al-Zahra and Shahid Beheshti hospitals in Isfahan in 2020–2021. After obtaining written consent from the parents of the infants, the study was approved by the ethics committee of Isfahan University of Medical Sciences with the code of ID IR.MUI.MED.REC.1399.869 and It has been registered on the clinical trial site as number IRCT20150423021910N7. Then 108 infants were selected according to the inclusion criteria and divided into two groups based on the random numbers table. The inclusion criteria were premature infants with gestational age of less than and equal to 32 weeks who feed with breast milk and Fortifier at the rate of one scoop per 25 cc. Infants’ total parenteral nutrition (TPN) was stopped during the first ten days of birth and they had received full enteral nutrition equal to 100 CC/kg/day.

Exclusion criteria were the presence of maternal vitamin D deficiency or diabetes, asphyxia at birth, receiving drugs affecting vitamin D metabolism such as diuretics, corticosteroids, anticonvulsant drugs, and intrauterine growth restriction of infants.

Then, the samples were selected among the eligible subjects and were randomly selected by permutation block methods with 4 blocks in each of the two groups.

In the first group, from the 7th day of birth, the consumption of daily 400 units vitamin D drops and in the second group, daily 600 units vitamin D drops was started. Based on the current protocols, tests of calcium and phosphorus, alkaline phosphatase and vitamin D serum levels were performed for the patient at the age of 5 weeks.

If the alkaline phosphatase level was above 1000, the wrist radiography was requested and the changes were reported by the radiologist.

The prescription of different doses of vitamin D was done by a superspecialist, and the evaluation at the 5th week of birth and the recording of test results were done by a superspecialist assistant. By following up the patient and infants’ examination at the age of 5 weeks, the superspecialist assistant checked the clinical symptoms of rickets (Wide fontanel, Wide wrist, Hypotonia, and Craniotabes) and registered the information in a form as well as Laboratory and radiological results. The neonatal superspecialist assistant and the radiologist did not know about the prescribed dose of vitamin D.

Wide fontanel refers to the size of the anterior fontanel greater than 3 x 3 cm. Craniotabes means excessive softening and thinning of a part of the skull bones. These changes may occur in the course of rickets.
For the infants with disorders in the initial examinations or clinical symptoms of rickets, the treatment with calcium and phosphorus salt was started and the tests were repeated weekly and the follow-up continued.

Quantitative variables were reported with mean (median) and standard deviation (interquartile range) and qualitative variables with frequency and percentage. The numerical variables between the two groups were compared with two-paired independent t-test and analysis of covariance in case of confounders. Comparison of qualitative variables between the two groups was done with chi-square test and Fisher's test.

Normality of continuous data was evaluated using Kolmogorov-Smirnov test and Q-Q plot. Non-normally positive skewed data were subjected to logarithmic transformation. Continuous and categorical data are presented as mean ± Standard Deviation (SD) and frequency (percentage). Normality of continuous data was evaluated using Kolmogorov-Smirnov test and Q-Q plot. Non-normally positive skewed data were subjected to logarithmic transformation. Continuous baseline variables were compared between two groups by independent samples t-test. Continuous main variables (Total calcium, ALP, PH, OH.D3) were compared between study groups by independent samples t-test in crude model and for adjusting the effect of confounding variables by analysis of covariance (ANCOVA) in adjusted models. All statistical analysis were performed by using SPSS version 26 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).

**Results**

The present study was conducted on 108 premature infants with a gestational age of less than and equal to 32 weeks who were born in Shahid Beheshti and Al-Zahrai Hospital affiliated to Isfahan University of Medical Sciences between 2021–2022. Infants were divided into two groups of 54 people (the first group received daily 400 units vitamin D and the second group received 600 units vitamin D daily from the 7th day of birth). The mean gestational age in the group of daily 400 units vitamin D was 28.40 ± 1.96 and in the group of daily 600 units vitamin D was 29.79 ± 1.34 and a significant difference was observed between the two groups (p < 0.001). The mean of birth weight in the group with daily 400 units of vitamin D was 1078.05 ± 271.41 and in the group with daily 600 units of vitamin D was 1224.53 ± 209.92 grams, and the difference between the two groups was significant (p = 0.002). In terms of gender, there were 26 (48.1%) boys and 28 (51.9%) girls in the group of 400 units vitamin D, and 29 (53.7%) boys and 25 (46.3%) girls in the group of 600 units vitamin D. No significant difference was observed between the two groups (p = 0.56). On the other hand, there was a significant difference between the two groups in terms of gestational age and birth weight (P < 0.05) (Table 1).

Table 1 shows the demographic information of the studied population.
Table 1
The mean of demographic information in two groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>600; IU/day</th>
<th>400; IU/day</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>29(53.7%)</td>
<td>26(48.1%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Female</td>
<td>25(46.3%)</td>
<td>28(51.9%)</td>
<td></td>
</tr>
<tr>
<td>Gestational Age; week</td>
<td>29.79 ± 1.34</td>
<td>28.40 ± 1.96</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Birth Weight; gr</td>
<td>1224.53 ± 209.92</td>
<td>1078.05 ± 271.41</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Infants were evaluated for clinical symptoms at the age of 5 weeks. The frequency of hypotonia was 0 case (0%) in the group of 400 units vitamin D and 1 case (1.9%) in the group of 600 units vitamin D daily and no significant difference was observed between the two groups (p > 0.99).

The frequency of wide fontanel was 0 case (0%) in the group of 400 units vitamin D and 1 case (1.9%) in the group of 600 units vitamin D and no significant difference was observed between the two groups (P > 0.99). The frequency of Craniotabes and Wide wrist was 0 in both groups and there was no significant difference (P > 0.05) (Table 2).

Table 2 shows the comparison of clinical symptoms in two groups.

Table 2
Comparison of clinical symptoms in the two groups

<table>
<thead>
<tr>
<th>Dose</th>
<th>Hypotonia</th>
<th>Wide fontanel</th>
<th>Wide wrist</th>
<th>Craniotabes</th>
</tr>
</thead>
<tbody>
<tr>
<td>600; IU/day</td>
<td>1(1.9%)</td>
<td>1(1.9%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>400; IU/day</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
</tr>
<tr>
<td>P value</td>
<td>&gt; 0.99</td>
<td>&gt; 0.99</td>
<td>0.000</td>
<td>0.000</td>
</tr>
</tbody>
</table>

In terms of radiologic criteria, wrist x-ray examination was done by the radiologist in cases of alkaline phosphatase above 1000 at 5 weeks of age; regarding bone fractures and couping ,widening frequency was 0 cases (0%) in both groups. Fraying was 2 cases (3.7%) in the 400 units vitamin D group and 1 case (1.9%) in 600 units group, and there was no significant difference between the two investigated groups (P > 0.99). In terms of reduction in bone density, there were 4 cases (7.4%) in the group of 400 units and 3 cases (5.6%) in the other group, and the difference between the two groups was not significant (P > 0.99) (Table 3).
Regarding the laboratory symptoms, the level of calcium at the age of 5 weeks was $9.93 \pm 0.88$ in the group of 400 units and $9.51 \pm 0.89$ in the group of 600 units. If the effect of confounders such as gestational age and birth weight was not removed, the level of calcium was significantly higher in the first group ($p = 0.016$).

By adjusting the effect of confounders using statistical methods, the difference between the two groups was weakly significant ($P = 0.062$)). Regarding the phosphorus level, it was $5.62 \pm 1.27$ units in the group of 400 units and $6.20 \pm 0.90$ in the other group. In this case, if the effect of confounders was not removed, the level of phosphorus was significantly higher in the second group ($P = 0.008$) and after adjusting the effect of confounders, the difference between the two groups was slightly significant ($P = 0.062$).

The level of alkaline phosphatase in the group of 400 units vitamin D was $871.00 \pm 366.44$ and in the group of 600 units vitamin D it was $792.44 \pm 321.99$, with no significant difference between the two groups (with and without adjusting the effect of confounders). The level of significance with and without adjusting the effect of confounders was $P = 0.596$ and $P = 0.239$ respectively.

The amount of vitamin D in the group of 400 units vitamin D was $37.51 \pm 14.4$ and in the group of 600 units vitamin D it was $57.77 \pm 24.79$, and a significant difference was observed between the two groups (with and without adjusting the effect of confounders) ($P < 0.001$) (Table 4).

## Discussion
osteopenia of prematurity is one of the premature infants diseases that can be associated with long-term physical and developmental complications. Vitamin D deficiency is common in preterm infants and caused by the lack of proper transfer of vitamin D through the placenta (usually occurs after 32 weeks), insufficient enteral nutrition, and lack of exposure to ambient light due to long-term hospitalization. Evaluation of premature infants at risk of osteopenia of prematurity is done by screening total or ionized calcium, phosphorus and alkaline phosphatase activity (5).

When these laboratory values are clearly abnormal, a wrist plain radiography performed often to evaluate the bones for osteopenia, rickets, or possible fractures. Usually, when serum alkaline phosphatase is more than 800IU/L, radiographic evaluation is recommended. It is true especially if serum phosphorus is less than 4.5mg/dl at the same time (6). Some sources have reported that alkaline phosphatase greater than 800–1000 IU/L or clinical evidence of fracture should be associated with radiographic evaluation of rickets (1).

Taking vitamin D supplements during pregnancy is associated with an increase in the amount of 25-hydroxyvitamin D in cord blood. According to the research findings, infants born to mothers who received vitamin D supplements had more weight and height than infants whose mothers did not (7). Moreover, infants born to mothers with severe vitamin D deficiency were smaller in height and weight, and their head circumference was smaller than infants born with normal vitamin D levels (8).

In addition, insufficient levels of vitamin D in cord blood were associated with the prevalence of severe respiratory tract infections in the first year of life and decreased bone density in childhood (10, 9).

In general, the condition of vitamin D in mothers is very inappropriate worldwide, especially in countries where vitamin D supplements are not used, there is little sunlight exposure and they wear a lot of clothes or have very dark skin, so the level of vitamin D deficiency in cord blood (less than 20 nmol/l) in European societies may be up to 70% (1). Vitamin D is important for supporting a large number of physiological processes such as bone mineralization, immunomodulatory functions, and early lung development. Additionally, recent studies have indicated that low vitamin D levels predispose to an increased risk of rickets, immune system dysfunction, asthma, type 1 diabetes, and respiratory tract infections in infancy (12, 11). Moreover, vitamin D is vital for cellular absorption of calcium that is done through the active form of vitamin D (1.25 dihydroxy vitamin D). Vitamin D is converted into 25-hydroxyvitamin D in the liver, and then transferred to the kidney, where it is converted into 1 and 25-dihydroxyvitamin D.

Furthermore, calcium absorption is mainly paracellular in the first weeks of life in premature infants and probably in term infants, and it is not dependent on vitamin D (13). Vitamin D depended absorption of higher calcium level may not happen until one to two months, in fact the exact time is not known (14).

Absorption of higher amounts of calcium dependent on vitamin D may not happen for one to two months, but the exact time is not known (14).
Vitamin D may interfere with drugs such as antibiotics and glucocorticoids that are used to treat sepsis and BPD. Moreover, all premature infants need vitamin D to prevent premature rickets, but recommended amount is different in various protocols (17, 16). The studies conducted in Europe have recommended a daily dose of 800–1000 IU vitamin D (14). However, based on the recent recommendations of the European Association for Gastroenterology, the required amount of vitamin D is 800 to 1600 IU per day (19, 18). In other sources, the recommended dose is from 400 to 1000 IU per day (21, 20). According to the protocol of the American Academy of Pediatrics for infants, the recommended amount of vitamin D per day is 400 units (22).

Considering the effective role of vitamin D in the prevention of osteopenia of prematurity and given that all three radiological, clinical, and biochemical criteria have been evaluated simultaneously in limited studies, this study was conducted with the aim of comparing the different doses of vitamin D (400 IU daily and 600 IU daily) in premature infants with ≤ 32 weeks of gestation. The infants under 32 weeks were divided into two equal groups and their gestational age, birth weight, and gender were checked. In terms of gestational age and birth weight, the difference between the two groups was significant, and therefore, in order to eliminate any possible confounding factor with statistical methods, the effect of these confounders was adjusted.

The amount of calcium measured at the age of 5 weeks in the 400 units group was slightly higher than the other group.

Without removing the effect of confounders, this difference between the two groups was significant. However, by removing this effect, the difference in calcium levels between the two groups was slightly significant. The measured amount of phosphorus in the group of daily 600 units vitamin D was more than the other group. Without removing the effect of confounders, the difference between the two groups was significant, while with the removal of this effect, the difference in the level of phosphorus between the two groups was slightly significant. In the present study, the amount of vitamin D at the age of 5 weeks in the group of 600 units was higher than the group of 400 units vitamin D. Furthermore, the level of alkaline phosphatase was higher in the group of 400 units. However, this difference was not significant and therefore there was no difference between the two groups.

The World Health Organization (WHO) has recommended a dose of 400–1000 units per day for premature infants (23). According to the clinical guidelines of the Endocrine Society, the amount of vitamin D required for infants, from birth to one year of age, is 400–1000 units per day (24). In some sources, for infants who are breastfed or those who use less than 1 liter of formula per day, the daily amount of vitamin D is 400 units per day (25). Some sources have recommended the amount of vitamin D needed from birth to 6 months as 400 units per day (26). In other sources, the amount of vitamin D in premature infants with a birth weight of less than 1500 gr is 200–400 units per day and in premature infants with a birth weight of more than 1500 gr, 400–1000 units per day are recommended (10). According to the protocol of the American Academy of Pediatrics for infants, the recommended amount of vitamin D is 400 units per day (22, 27).
So far, various studies have been conducted on the effect of vitamin D in the prevention of osteopenia of prematurity. In a study conducted in America by Anderson et al., it was found that taking a daily dose of 800 units of vitamin D for infants less than 32 weeks improved the level of 25-hydroxyvitamin D at 4 weeks, bone density, and linear growth (28). In a study by Alizadeh Taheri et al. (2013), 60 premature infants were examined. One group received daily 400 units of vitamin D and the other group received 200 units, but there was no difference between the levels of calcium, phosphorus and alkaline phosphatase in the two groups (29).

In terms of clinical criteria, there was no difference in wide fontanel and wide wrist in Taheri's study, which was consistent with the present study. Besides, in terms of radiological criteria, there was no difference between the two groups, which was in line with the present study.

In a study conducted by Yang et al. (2018), no difference was observed between the amounts of calcium, phosphorus and vitamin D in a high dose (800–1000 units per day) and a low dose of vitamin D (400 units per day) (30), while the results related to the height and head circumference growth and immune system function were better in the higher dose group.

In a study by Alizadeh et al. (2006), premature infants under 38 weeks with a birth weight of less than 2000 gr were evaluated, and one group was treated with 400 units of daily vitamin D and the other group was treated with 1000 units. The results showed that the mean level of calcium, phosphorus and alkaline phosphatase was not significantly different in two groups. Moreover, none of the two groups showed clinical or radiological symptoms of rickets, so a lower dose of vitamin D was suggested to prevent osteopenia of prematurity. (31). In the present study, there was no difference in the occurrence of clinical or radiological symptoms of rickets between the two groups, which was in line with the study of Alizadeh et al.

DEXA method is used to evaluate bone mineral content, but this method is only possible in a number of centers and it is expensive (1, 32).

Standard X-ray films are not an accurate assessment of bone demineralization because the bone mineral content (BMC) must decrease by 20%-30% or more to be detected by this method (3). However, they can detect Bone FX, Osteopenia, Couping, Widening and Fraying (3). Wrist or knee radiography in the 4th to 5th week of birth or afterward in high-risk infants is a practical method in the case of clear rickets (1).

Backstrom et al. used two different doses of vitamin D (200 and 960 daily units) in premature infants up to the age of three months and checked the bone mineral content using the DEXA method, and no difference in the incidence of premature osteopenia was seen in these two groups (33).

**Limitations**

One of the limitations of this study was the small sample size in each week of gestational age, which was too small to generalization. It is recommended to do similar studies with a larger sample size.
Conclusion

According to the results of this study, after adjusting the effect of confounders such as birth weight and gestational age between the two investigated groups in premature infants ≤ 32 weeks of gestation, the amount of calcium in the group of daily 400 units vitamin D was more than the group of 600 units but this difference was weakly significant. Also, the level of phosphorus in the group of 600 units vitamin D was higher than the group of 400 units vitamin D, and this difference was slightly significant. The level of alkaline phosphatase was not significantly different between the two groups. Also, vitamin D level was significantly higher in the group of 600 units, but no difference was observed between the two groups in terms of clinical and radiological symptoms of rickets.

Declarations

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgment

This article was supported by vice chancellor of Isfahan University of Medical Sciences. The kind support of the relevant people in this field is greatly appreciated.

Funding

The present research was carried out with the financial support of Isfahan University of Medical Sciences.

Author information

Authors and Affiliations

Department of Pediatrics, Division of Neonatology, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Behzad Barekatain

Department of Pediatrics, Division of Neonatology, Isfahan University of Medical Sciences, Isfahan, Iran.

Shima Hamidipour

Department of Pediatrics, Division of Neonatology, Child Growth and Development Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

Zohreh Badiei
Department of Radiology, Isfahan University of Medical Sciences, Isfahan, Iran.

Maryam Farghadani

Contributions

Substantial contributions to the conception or design of the work: B.B and Z.B. Analysis and interpretation of data for the work: SH.H. Drafting the work or revising it critically for important intellectual content: BB and M.F. Final approval of the version to be published: B.B

Corresponding author

Correspondence to Behzad Barekatain

Ethics declaration

Ethics approval and consent to participate

the study was approved by the ethics committee of Isfahan University of Medical Sciences with the code of ID IR.MUI.MED.REC.1399.869 and It has been registered on the clinical trial site as number IRCT20150423021910N7.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References


