Iranian J Publ Health, 2006, Vol. 35, No. 3, pp.58-63

A Randomized Clinical Trial of Prophylactic Effects of Vitamin D on Different Indices of Osteopenia of Prematurity

**P Alizade [1], F Naderi [1], K Sotoudeh [2]*

[1]Dept. of Pediatrics, Bahrami Children Hospital, Tehran University of Medical Sciences, Iran
[2]Research Development Center, Bahrami Children Hospital, Tehran University of Medical Sciences, Iran

(Received 2 Jan 2006; revised 10 Jun 2006; accepted 25 July 2006)

Abstract

Prevention and treatment of the rickets of prematurity is an important aspect of the care of preterm infants. The purpose of this study was to compare the prophylactic effects of different doses of vitamin D on the clinical, biochemical and radiological indices of the rickets of prematurity. In a randomized clinical trial, 68 premature infants (<38 weeks) with birth weight under 2000 g, randomly divided in two groups. Infants received 400 IU/d vitamin D in Group A (n=32) and 1000 IU/d in group B (n= 36). On the 9th week of birth, serum calcium, phosphate, and alkaline phosphatase were measured and x-ray of left wrist and physical examination were performed. The average serum calcium, phosphate and alkaline phosphatase in both groups had no difference (*P*= 0.326, 0.466, 0.147, respectively) and no one had a radiological or clinical picture of rickets. In conclusion we recommend low dose vitamin D for prevention of the osteopenia of prematurity.

Keywords: *Premature infant, Rickets, Vitamin D, Iran*

Introduction

Premature infants are known to be at risk of developing metabolic bone disease (Rickets or osteopenia), and this risk is inversely related to the infant's birth weight. Reports of osteopenia of prematurity are increasing due to the improved survival rates of low birth weight infants. The reported rates of rickets among the very low birth weight infants (Under 1500 g) and infants with birth weight under 1000 g are 30% and 50%, respectively (1). In premature infants with gestational age under 32 wk, bone mineral content (BMC) was found to be 25% to 70 % lower than term infants (2).

The etiology is multi factorial and includes phosphorous, calcium and vitamin D deficiency; certain drugs, and immobilization (3). When premature babies are fed with human milk, both the calcium and phosphorous supplies are insufficient; only 25% of amounts needed for normal bone mineralization; but the critical factor leading to rickets is lack of phosphorous (3, 4). Thereafter serum phosphate levels decrease and there is not enough substrate to incorporate into the organic bone matrix (5). Biochemically, rickets is characterized by an increase in serum levels of alkaline phosphatase (1, 5). Clinical rickets usually appears between the 6th and 12th postnatal week and a wrist x-ray film at 6 to 8 wk of age remains a practical assessment of the presence of overt rickets (1). To prevent rickets of prematurity, vitamin D, calcium and phosphorous supplementation is needed. The recommendations for vitamin D supply are different in Europe and America. The European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) recommend a dose of 800-1600 IU/d (6), while The Ameri-

***Corresponding author:** Tel: +98 21 77556969, Fax: +98 21 77551584, E-mail: bahrami_ch@yahoo.com

can Academy of Pediatrics (AAP) recommends only 400 IU/d (7). Some studies indicated that a daily vitamin D dose of less than 400 IU/d sufficient to maintain vitamin D status and activity normal, but high dose of vitamin D may cause hypervitaminosis D which involves a risk of hypercalcemia with subsequent complications (1).
As far as we know, in Iran, studies comparing the effects of low dose versus high dose vitamin D on biochemical, and radiological indices and clinical manifestations of osteopenia in premature infants have not been performed before; therefore this study was conducted to compare these effects.

Materials and Methods

In this randomized clinical trial we compared the effects of low dose versus high dose vitamin D on biochemical, radiological and clinical manifestations of rickets of prematurity. Ninety six preterm infants-inpatients or outpatients-presented in Bahrami Children Hospital, Tehran, Iran from May 2001 to May 2002, were enrolled in the study. The inclusion criteria were gestational age under 38 wk and birth weight less than 2000 g. The exclusion criteria were use of specific medications interacting with vitamin D metabolism (eg: Anticonvulsants, diuretics, corticosteroids and so on) in mother, diabetes mellitus in mother, previous IUGR or SGA baby, chronic use of fursemide in infant and being NPO (non per os) for more than 2 wk. The withdrawal criteria were failure of taking vitamin D supplements according to the protocol of the study and failure to follow orders like performing radiography. Of 96 preterm infants, only 68 infants performed the orders (x-ray and laboratory test) in the 9th wk. Gestational age was determined based on the history of mothers' last menstrual periods, prenatal sonographic findings and postnatal physico-neurological examination. Within 2 wk of birth, eligible infants randomly divided in two groups by block randomization of two, to receive a vitamin D 400 IU/d (group A) and 1000 IU/d (group B) from the time they tolerated full enteral nutrition until they gained a normal term birth weight (3000 to 3500 g).The dose of vitamin D in both groups kept constant.
All parents received written instructions about the purpose and protocol of the study after signing an informed consent form which was approved by the local institutional review board for human investigations. Infants who could tolerate breast milk, received calcium supplement as calcium gluconate (90-120 mg/kg/d) and phosphate supplement as phosphate sandose, effervescent tablet (55-75 mg/kg/d). For infants deprived human milk, premature formula (Prenon), enriched in calcium and phosphate, was calculated and prescribed.
In the 9th wk, plasma ionized calcium, serum inorganic phosphate and alkaline phosphatase were measured, x-ray of left wrist and a through physical examination were carried out.
Ionized calcium and phosphate were analyzed using spectrophotometry and serum alkaline phosphatase activity was measured using paranitrophenol reaction. Physical examination and x-ray analysis were made blind by expert pediatricians. Diagnosis of premature osteopenia was based on abnormal radiographs (osteopenia, bone fractures, intracortical resorption) and biochemical picture including normal serum calcium, low serum phosphate and high serum alkaline phosphatase concentrations with or without signs and symptoms of rickets (Craniotabes, rickets rosary, wide fontanel, Harrison groove, kyphosis/scoliosis, Potts belly).
Statistical analyses were performed by SPSS version 10.1, software package (SPSS Inc, Chicago, IL), using Chi- square and student *t*-test. *P* values less than 0.05 were considered statistically significant. Based on our pilot study on preterm infants, we considered our data for estimating the sample size. The total sample size of 60 infants randomized in 2 groups provided 80% statistical power to detect 60 units of serum alkaline phosphatase and 0.4 mg/dL of phosphorous and calcium difference at the alpha level of 0.05 and beta level of 0.2. We also

increased the sample size to 96 for considering drop out or loss of follow up cases.

Results

Sixty eight infants- 32 in group A and 36 in group B- completed the study. Distribution of sex in both groups were the same (Male= Female). The youngest infant entered in this study was 4 d old in group A and 5 d old in group B and the oldest in both groups was 13 d old. Sixty five infants had gestational age between 29-38 wk and 3 were under 29 wk. The birth weights of 59 infants were between 1500-2000 g and 9 had birth weight under 1500 g. There was no significant difference between birth weight in the two groups (*P*= 0.94). The groups' characteristics and anthropometric results are given in Table 1.

The average duration of taking vitamin D supplements in group A was 46.7 d and in group B 47.5 d (*P*= 0.438).The average serum calcium, phosphate and alkaline phosphatase in the ninth week after birth in both groups were in normal range and there were no significant differences between the groups (*P*=0.325, 0.466, 0.147, respectively) (Table 2). None of the infants had rickets picture in x-ray. Only 4 infants (two in each group) had wide fontanel (more than 3×3 cm) without other manifestations of rickets in physical examination. Thyroid test performed in these 4 infants and hypothyroidism detected in only one of them and the remained three, had no additional findings.

Table 1: Group characteristics and anthropometric results in preterm infants receiving different doses of vitamin D

	Group A (400 IU /d)* n=32	**Group B (1000IU/d)* n=36**	***P***
Gestational age (wk)	32.8 (28-37)	32.7 (28-37)	0.851
Birth weight (g)	1736 (1300-1950)	1739 (1100-1950)	0.946
Age of starting supplementation (days after birth)	10.6 (4-13)	11.1 (5-13)	0.254
Duration of supplementation (days)	46.6 (35-56)	47.4 (40-56)	0.438

**Mean (Min- Max)*

Table 2: Comparison of serum calcium, phosphate and alkaline phosphatase activity in infants on the 9th week of age

	Group A (400 IU/d) * n=32	**Group B (1000IU/d)* n=36**	***P***
Serum calcium(mg/dL)	9.83 (9.10-10.7)	9.71(8.9-10.7)	0.326
Serum phosphate(mg/dL)	5.55 (4.4-6.8)	5.44 (4.3-6.3)	0.466
Serum alkaline ahosphatase (IU)	602.5 (420-750)	633.3 (415-753)	0.147

**Mean (Min- Max)*

Discussion

Clinical rickets has been recognized as a common complication of prematurity for more than forty years, and nowadays is seen more commonly. In preterm infants fed unsupplemented human milk, bone mineral content measurements remain significantly lower than the corresponding intrauterine values (1-3). Chen et al. (8) studied the prevalence of rickets during subjects' first 18 months of life and found that premature infants in comparison with term infants (=>37 wk gestation) were more likely to have rickets, 9.4% versus 5.2%, respectively. Oyatsi et al. (9) found the incidence of rickets of prematurity by 6 months of age to be 58.8 %. Radiological changes characteristics for rickets have been found in 55% of infants with a birth weight of less than 1000 g and in 23% of infants less than 1500 g at birth (3). Bone Mineral Content (BMC) can be measured by DEXA (Dual Energy X-ray Absorptiometry) but in routine clinical practice it is performed in few centers and has its own 'baggage', such as cost, availability and logistic difficulties (1). It is usually said that standard x- ray films are not an accurate assessment for bone demineralization because BMC must decrease by 30% or more to be diagnosed by this method , however standard x-ray films can detect fractures and a wrist x-ray film at 6 to 8 weeks of age remains a practical assessment of the presence of rickets(1).

In this study we have shown that low dose of vitamin D intake, has the same results in bone mineral content as high dose vitamin D, recommended by ESPGAN. We used biochemical, radiological and clinical criteria to diagnose rickets of prematurity and there were no significant differences between the groups for biochemical and radiological features, as well as clinical manifestations. Simple biochemical indicators of bone mineralization such as serum alkaline phosphatase, and to some extent, serum phosphate and serum calcium has been suggested to be an easy way of identifying metabolic bone disease in premature infants. Kovar et al. (10) introduced use of serum alkaline phosphatase level for screening rickets of prematurity. They suggested a cut off level of alkaline phosphatase activity above 5 times the upper limit of the normal adult reference rate (> 1200 IU) as an indicator of rickets. A radiographic study (11) found a significant association between serum phosphate level and bone mineralization. In our present study we used these biochemical and radiological criteria and our results were consistent with these studies. In a study (12), the association between serum alkaline phosphatase and serum phosphate with bone mineral content by using DEXA in premature infants was negative. In another study, (13) a positive association between alkaline phosphatase and phosphate and BMC was found. Comparison of these studies is difficult because the measurements of biochemical variables and BMC are inconsistence. Based on the previous studies (14, 15), ESPGAN recommends a vitamin D dose of 800-1600 IU/d for preterm infants. It is suggested that a vitamin D dose of 400 IU/d is insufficient and that vitamin D requirements in preterm infants are at least three times higher. In our study we questioned the ESPGAN high dose vitamin D recommendation as it seems to be merely a recommendation and not an established requirement based on clinical trials. On the other hand the AAP has recommended a vitamin D dose of 400 IU/d for preterm infants. Koo et al. (16) showed that even as little as 160 IU/d of vitamin D for 24-29 ds maintain normal and stable vitamin D status in preterm infants who receive adequate mineral intake. They could not investigate the effect of low dose of vitamin D on the bone mineral density because DEXA measurements were not available.

Backstrom et al. (17) compared the effects of low (200 IU/kg/d) and high (960 IU/kg/d) doses of vitamin D on bone mineral accretion, using DEXA, in two small groups of premature infants until 3 months old. They noticed that there were no differences in bone mineral content

and in bone mineral density at 3 and 6 months corrected age between the infants receiving low or high dose vitamin D. They also measured serum 1,25-(OH)2 D concentration at birth, 6, 12 and 24 wk and the levels in both groups were within the reference limits, indicating sufficient 1-hydroxylation of vitamin D even with a low dose of vitamin D.

Two limitations of our present study were that we neither measured serum metabolites of vitamin D nor using DEXA for evaluating BMC. Vitamin D mainly stimulates absorption of intestinal calcium and phosphorus and also is a potent factor in bone resorption. Hypervitaminosis D involves a risk of hypercalcemia, hypercalciuria, polyuria, dehydration, hypertension, stones in the lower urinary tract and metastatic calcification; therefore vitamin D should not be given in excess to preterm infants (18). In our study 4 infants (two in each group) had wide fontanel (larger than 3×3 cm), however they did not have other manifestations of rickets in physical examination and their biochemical and radiological tests were normal. Thyroid tests performed for them and hypothyroidism detected in only one of them and the other three, had no important findings, so prematurity assumed to be the main cause.

In conclusion we recommend 400 IU/d of vitamin D supplement for premature infants until they gain a normal term birth weight (3000-3500 g); this low dose of vitamin D as well as high dose of vitamin D can prevent osteopenia of prematurity.

Acknowledgements

The authors thank Dr Patricia Khashayar for help in editing article, and also like to extend their thanks to Mrs Olya Ghobady and Mrs Zohre Jalili Tahmasebi for their secretarial assistance.

References

1. DeMarini S, Tsang RC (2002). Disorders of calcium, phosphorus and magnesium metabolism. In: *Neonatal- Perinatal Medicine*. Eds, Fanaroff and Martin. Mosby. 7th ed. St. Louis. USA .pp:1389-92.
2. James JR, Congdon PJ, Truscott J, et al. (1986). Osteopenia of prematurity. *Arch Dis Child*, 61: 871-76.
3. Backstrom MC, Kuusela AL, Maki R (1996). Metabolic bone disease of prematurity. *Ann Med,* 28: 275-82.
4. Faerk J, Petersen S, Peitersen B, et al. (2000). Diet and Bone Mineral Content at Term in Premature Infants. *Pediatr Res,* 47:148-56.
5. Rauch F, Schuenau E (2002). Skeletal development in premature infants: A review of bone physiology beyond nutritional aspects. *Arch Dis Child Fetal Neonatal Ed,* 86: 82-5.
6. ESPGAN Committee on nutrition of the preterm infant (1987). Nutrition and feeding of preterm infants. *Acta Pediatr Scand*. suppl, 336: 1-14.
7. AAP Committee on Nutrition (1985). *Pediatric Nutrition Handbook*. Elk Grove Village, Ill: American Academy of Pediatrics.pp: 25-7.
8. Chen Y (1994). Prematurity as a predictor of rickets in Shanghai infants. *Public Health*, 108:333-39.
9. Oyatsi P, Musoke RN, Wasunna AO (1999). Incidence of rickets of prematurity at Kenyatta National Hospital, Nairobi. *East Afr Med J,* 76: 63-6.
10. Kovar I, Mayne P, Barltrop D (1982). Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. *Lancet*, i: 308–10.
11. Koo WWK, Gupta JM, Nayanar VV, et al. (1982). Skelet al changes in preterm infants. *Arch Dis Child,* 57: 447-52.
12. Faerk J, Peitersen B, Petersen S, et al. (2002). Bone mineralization in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. *Arch Dis Child Fetal Neonatal Ed,* 87: F133-F136.

13. Ryan SW, Truscott J, Simpson M, et al. (1993). Phosphate, alkaline phosphatase and bone mineralisation in preterm neonates. *Acta Pediatr,* 82: 518-21.
14. Glorieux FH, Salle BL, Delvin EE, David L (1981). Vitamin metabolism in preterm infants: Serum calcitriol values during the first five ds of life. *J Pediatr*, 99: 640-43.
15. Salle BL, Glorieux FH, David L, Meunier G (1983). Vitamin metabolism in preterm infants: Serial serum calcitriol values during the first five ds of life. *Acta Pediatr Scand*, 72: 203-6.
16. Koo WW, Krug-Wispe S, Neylan M, Succop P, Oestreich AE, Tsang RC (1995). Effect of three levels of vitamin D intake in preterm infants receiving high-mineral containing milk. *J Ped Gastroenterol Nutr*, 21: 182-89.
17. Backstrom MC, Maki R, Kuusela AL (1999). Randomised controlled trial of vitamin D supplementation on bone density and biochemical indices in preterm infants. *Arch Dis Child Fetal Neonatal Ed*, 80:161-66.
18. Chesney RW (1990). Requirements and upper limits of vitamin D intake in term neonate, infant and older child. *J Pediatr,* 116:159-166.