ORIGINAL ARTICLE

The effect of calcium and phosphorus supplemtation on metabolic bone disorders in premature infants

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Abstract

Objective: To determine the impact of calcium and phosphorus on radiological and biochemical characteristics of osteopenia in premature infants.

Methods: The randomised clinical trial study was conducted at Valie-e-Asr Hospital of Zanjan city, Iran, from December 2010 to June 2011. It involved monitoring 40 premature neonates over a period of six months. The babies, who were fed with breast milk and 400 units of vitamin D daily, were randomly divided into two equal groups. One group received supplement of calcium and phosphorus. Serum calcium, phosphorus and alkaline phosphatase levels as well as growth parameters (including weight, height, and head circumference) were measured every two weeks. At the end of the study, wrist X-ray was done for evaluation of osteopenia. Data was analysed using SPSS 16.

Results: Radiological changes, characteristic of osteopenia, were found in 8(40%) cases and 13(65%) controls ($p<0.113$). The mean of weight, length and head circumference increased significantly from second to sixth week during follow-up ($p<0.0001$). Phosphorus and alkaline phosphatase activity decreased significantly from second to sixth week of follow-up ($p<0.02$, $p<0.01$ respectively). However, repeated measurement analyses did not show significant effect of intervention in biochemical and growth parameters in the trial group.

Conclusion: The study didn't show significant effect of calcium and phosphorus on prevention of osteopenia and improvement of growth. Further studies of longer duration and with different doses of supplement are recommended.

Keywords: Osteopenia, Prematurity, Calcium, Phosphorus. (JPMA 64: 635; 2014)

Introduction

Metabolic bone disease (rickets and osteopenia) is a common problem in preterm infants. This is reversely related to birth weight and age of newborns. The prevalence of osteopenia is rising with the increase in the survival rates of low birth weight (LBW) (birth weight less than 2500g) and very low birth weight (VLBW) (birth weight less than 1500g) newborns.[1] In preterm newborn with gestational age below 32 weeks, the mineral content of bone is 25%-70% less than term neonates.[2]

Osteopenia could be found by reduced density in stereotype X-rays. However, subclinical disease might be very common and is defined by increased serum alkaline phosphates levels.[3]

Calcium absorption depends on vitamin D status, solubility of calcium salts, and quality and quantity of lipid achievement. Moreover, in preterm babies, bone mineral demands are influenced by body contents at birth which depends on the duration of gestation and maternal vitamin intake.[4]

There are several causes for this defect such as vitamin D, calcium and phosphorus deficiency, prescription of lexis, contamination of some total parenteral nutrition (TPN) solution with aluminium during the treatment and immobilisation. This defect is also found in several diseases, including respiratory diseases, neonate sepsis and liver diseases.[3,5] Newborns receive 80% of bone calcium, phosphorus and magnesium through an active transfer from the placenta in the last three months of pregnancy. Thus, newborns with low birth weight and nutrients intake (calcium, phosphorus and vitamin D), sometimes with prolonged period of total parenteral nutrition and immobilisation especially have a higher risk for appearance of metabolic bone diseases.[6-9] However, some investigations did not show the beneficial effects of feeding on the prevention of metabolic bone disease.[10-12]

In Iran, few studies about fortification of human milk with calcium and phosphorus for improvement of bone mineral content have been carried out. Therefore, this study was conducted to investigate the effect of calcium and phosphorus supplementation on the prevention of osteopenia and improvement of growth in preterm infants.

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Subjects and Methods

The randomised clinical trial was conducted in Valie-e-Asr Hospital of Zanjan City in Iran, from December 2010 to June 2011. It comprised 40 preterm newborns with gestational age of less than 37 weeks and birth weight less than 2500 grams, who were medically stable and were only breast-fed. Neonates with TPN and being nil per oral (NPO) for more than one week, receiving medications interfering with vitamin D metabolism (anticonvulsants, diuretics, corticosteroids etc.), those with sepsis syndrome, significant respiratory, neurologic, renal, genetic, cardiovascular, hepatic or gastrointestinal diseases as well as neonates whose mothers had osteomalacia, diabetes, parathyroid gland disease and renal disorders were excluded from the study.

The selected neonates were randomly divided into two groups. The trial group received calcium supplements as calcium gluconate 10%; 5ml/kg/day (45mg/kg/day of elemental divided 3 times a day), phosphorus as potassium phosphate 17%; 1 ml/kg/day (24 mg/kg/day divided in 12-hours to be taken orally) and vitamin D 400 U daily. The control group received only vitamin D 400 U daily. All neonates received supplements and/or vitamin D at the ages of 10 days to 6 weeks by mothers under the supervision of a qualified person who did not have any role in data analysis.

The study was approved by the institutional ethics committee of Zanjan University of Medical Sciences, and parents were asked to sign a written consent prior to their enrolment. All babies were followed up over a period of 6 weeks from the time of their birth by phone calls to their parents and were visited weekly for any medical complications such as hypotension, bradycardia, medications, diarrhoea and vomiting. At the end of the second, fourth and sixth weeks post birth, the infants' serum calcium, phosphorus, and alkaline phosphatase were measured. Ionized calcium was measured by Arcenazo method (Pars Azmoon, Karaj, Iran), Phosphorus was measured by phosphomolybdate U.V method (Elitech, Sees, France) and serum alkaline phosphatase activity by DGKC-method (Pars Azmoon, Karaj, Iran).[13]

A nurse was employed to measure the infants' length by using an infant measuring board to the next succeeding 0.1cm. Head circumference was measured at the largest occipito-frontal circumference by means of a non-stretchable paper tape to the next succeeding millimeter. Weight was measured with the use of a calibrated infant scale to the nearest 5g (725 Mechanical Beam Baby Scale). All measurements were performed at the end of the second, fourth and sixth weeks of therapy.

At the 6th week, an X-ray of left wrist accompanied by a thorough physical examination was done by an expert and a paediatrician without being informed about the research project. Diagnosis of premature osteopenia was based on abnormal radiographs (osteopenia bone fractures) and biochemical picture including normal serum calcium, low serum phosphate and high serum alkaline phosphates concentrations.

Kolmogorov-Smirnov test was used to evaluate the distribution of quantitative variables. Values were expressed as mean and standard deviation (SD) as the most appropriate among the other statistical procedures. Comparisons were performed by chi-square test for categorical variables, independent t-test and repeated measure analysis of variance (ANOVA) for quantitative variables. P less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 16.0.

Results

The distribution of gender in the two groups was similar ($p<0.752$).

The mean gestational age of the cases (33.2±1.7weeks) and the controls (33.8±1.8 weeks) was similar ($p<0.858$).

There were no significant differences in weight, length and head circumference between the two groups at the beginning of the study (Table-1).

Mean age of mothers in the trial group was 25.7±4.7 years and it was 26±4.7 years ($p<0.868$) in the control group. All neonates were exclusively breast-fed and had similar feeding patterns.

The mean of weight, length and head circumference increased significantly from the second to sixth week over the follow-up period on the basis of Huynh-Feldt or Sphericity test ($p<0.0001$, Table-2). Pair-wise comparisons of the three growth parameters for every two-week-interval was also significant as indicated by Bonferroni

Table-1: Characteristics of newborns at birth.

	Case	Control	P value
Number of newborns	20	20	
Male/ female	10/10	9/11	0.752
Maternal age, year	25.8± 4.7	25.7± 4.7	0.868
Mean Gestational age, week	33.3± 1.7	33.3± 1.8	0.858
Mean Weight, gram	1996.5±304.9	1881±308.7	0.106
Mean Height, cm	44.13 ± 1.12	44.28± 1.21	0.686
Mean Head circumference, cm	30.56± 0.98	30.44±0.89	0.681

SD: Standard Deviation.

Table-2: Values of weight, length and head circumference in premature neonates receiving supplements and in the control group from the 2nd to 4th weeks and from the 2nd to 6th weeks.

Growth	Second week	Forth week	Sixth week	F	P-value
Weight (gram)					
Trial	2125.5±268.2	2336.5±267.6	2621.5±289.7	224.7	<0.0001
Control	2073.0±391.4	2276.5±401.3	2483.0±411.8		
Length(cm)					
Trial	46.01±2.69	46.81±2.67	47.81±2.69	412.4	<0.0001
Control	45.67±2.79	46.34±2.71	47.04±2.78		
†Head C(cm)					
Trial	33.49±0.94	34.01±0.94	34.64±0.94	967.9	<0.0001
Control	33.30±1.03	33.79±1.04	34.31±1.09		

*Results were shown by Mean±Standard deviation. †Head circumference.

Table-3: Comparison of serum calcium, alkaline phosphatase and phosphorus in preterm neonates receiving supplementary and in controls on the, 2nd, 4th and 6th week of age.

Parameters	Second week	Forth week	Sixth week	F	P-value
Calcium(mg/dl)					
Trial	9.01±1.05	9.03 ±0.76	8.93±1.22	0.36	0.63
Control	8.47±0.97	8.51±0.72	8.39±1.12		
ALP					
Trial	522.85±81.76	500.35±73.77	483.00±68.53	5.13	0.01
Control	559.20 ±94.57	535.25±75.72	539.85±74.67		
phosphorus(IU)					
Trial	4.33±0.40	4.56±0.28	4.43±0.35	4.47	0.02
Control	4.35±0.49	4.57±0.37	4.36±0.57		

*Results were shown by Mean±Standard deviation. ALP: Alkaline Phosphatase.

test (p<0.0001 for all). However, the intervention did not have a significant effect on the growth parameters (p<0.44 for weight; p<0.54 for height; and p<0.43 for head circumference).

The mean level of calcium concentration did not alter during the follow-up, while the mean level of phosphorus concentration significantly changed (p<0.02; Table-3). Pair-wise comparisons showed a significant mean difference of phosphorus level throughout the second and fourth weeks using Bonferroni test (p<0.001). Alkaline phosphate activity decreased significantly from the second to sixth week of follow-up indicated by Huynh-Feldt test (p<0.01). In pair-wise comparisons, the mean level of alkaline phosphates was significantly different between the second and fourth weeks (p<0.02) and between the second and sixth weeks ((p<0.046). However, the intervention did not significantly affect the level of these variables (p<0.06 for calcium; p<0.91 for phosphorus; and p<0.06 for alkaline phosphates). Radiological changes, characteristic of osteopenia, were found in 8(40%) and 13(65%) cases and controls, respectively (p<0.113).

Cochran test pointed out a significant decrease in the number of infants with osteopenia throughout the study (p<0.0001).

Discussion

The study showed that adding supplement of calcium and phosphorus in premature infants did not have a significant effect on the rate of osteopenia. Some studies have shown similar results.[8-10] However, there were several studies that demonstrated the beneficial effects of supplementary feeding on the reduction of osteopenia and improvement of bone mineral contents.[2,5,9,14-16]

These contradictory results might be due to the prescription of different doses of supplements or minerals or the age of neonates at the time of study or other environmental factors.

One study showed positive effect of adding supplementary nutrients with different mineral contents in 3 groups of neonates in comparison to neonates who were only breast-fed. It found that different concentrations of minerals produced no significant changes in the intervention groups.[17] Within a few weeks

after birth, premature infants can absorb calcium and phosphorus in sufficient amounts and there is some evidence that physical activity programmes might promote moderate short-term weight gain and bone mineralisation in preterm infants.[2,18]

Two researches confirm that adding higher concentrations of calcium and phosphate to Troph Amin, an amino acid injection, while maintaining its solubility improved neonatal parentral nutrition and reduced the rate of metabolic bone disease.[19,20]

More recently, the Expert Panel of the Life Sciences Research Office of Paediatrics recommended that an adequate nutritional intake of calcium, phosphorus and vitamin D and passive physical exercises may reduce osteopenia during first weeks of life and may increase growth of preterm infants.[21]

In our study, the intervention group which received the complements of calcium and phosphorus showed significant changes in phosphorus and alkaline phosphate levels in the sixth week of study. However, our trial did not show any significant effect on calcium, phosphorus and alkaline phosphatase levels as the two groups of case and control were compared. This finding might be due to the short period of our study or prescribing only low doses of calcium and phosphorous supplements.

Evaluation of phosphorus and alkaline phosphatase is a useful method in the assessment of the risk of metabolic bone diseases, and periodical analysis in order to assess response to nutrients the treatment is recommended.[2] One study reported that adding calcium, phosphorus, zinc and selenium to breast-fed VLBW infants and premature infants efficiently improved calcium and phosphorus absorption and reduced alkaline phosphate level.[22] However, some studies do not assert the effectiveness of supplementary calcium and phosphorous on alkaline phosphatase levels.[12,18]

One study indicated that in premature infants administration of low doses of vitamin D had similar preventive effect than high doses of vitamin D.[23] In another study that compared human fortified milk with premature formula, both case and control groups had similar growth velocity and phosphorus and alkaline phosphate level, but calcium significantly was higher in newborns fed with human fortified milk.[24]

Another study showed that mineral accretion in preterm breast-fed infants who had taken phosphorus and calcium, plus vitamin D 1200 IU daily was significantly higher than the infants who received only vitamin D, and rickets was significantly more common in the group that did not receive phosphorus and calcium.[25]

In our study, the weight and linear and head growth of the intervention infants was significantly higher at the sixth week of the study. However, the trial had no significant effect on growth parameters. In agreement with our study, another study found that supplement of calcium and phosphorus intake in premature breast-fed infants led to a significant increase in the length and head circumference, but did not have any significant effect on the mean weight or crown-heel length.[14] Still, there are several studies that do not support the effectiveness of supplementary feeding on growth parameters.[9,15]

One study reported that fortified formula promoted growth in preterm infants.[12] In our study, all neonates were breast-fed. Considering that protein intake is an important factor for growth, lack of difference in growth parameters of the control and trial groups could be due to similar feeding patterns.

The contradictory results between our study and previous studies can be explained by demographic differences, the amount of vitamin D, calcium and phosphorus supplements prescription, duration or period of evaluating their impact, and the criteria used to assess metabolic bone disease.

Conclusion

The study did not provide any evidence to suggest the effectiveness of supplementary calcium and phosphorus in osteopenia of prematurity and growth of infants. Regarding the importance of taking preventive measures in giving care to premature newborns, further studies with different doses of supplements over a longer period of time are recommended to move towards the most effective protocol that may ensure positive outcome.

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