Bone Mineral Content Measured by DEXA Scan in Preterm Neonates Receiving Total Parenteral Nutrition with and without Phosphorus Supplementation

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Abstract: Intravenous phosphorus preparation was not available in Egypt till recently. So we aimed to prove the positive effect of adding intravenous phosphorus to Total Parenteral Nutrition (TPN) on Calcium (Ca) and Phosphorus (PO4) metabolism of preterm neonates by measuring Bone Mineral Content (BMC) using DEXA scan. A case-control study was conducted in NICU of Obstetric and Gynecology Hospital of Ain Shams University which is a tertiary care unit in Cairo. Thirty preterm infants were prospectively enrolled in the study divided into 2 groups; 15 preterm infants received TPN with phosphorus supplementation (group 1) and 15 preterm received TPN without phosphorus supplementation (group 2). Serum Ca, PO4, and Alkaline Phosphatase (ALP) assay were done together with urinary calcium/creatinine (Ca/Cr) ratio, abdominal ultrasound and DEXA scan. There were no significant differences regarding serum Ca and PO4 between group 1 and 2 Yet there were highly significant increase in serum ALP and urinary Ca/Cr ratio in group 2 compared to group 1 (p = 0.001). Also group 1 had significantly higher BMC compared to group 2 even with TPN duration less than 15 days (p = 0.001). BMC was significantly positively correlated with G.A and B.W in both groups and was significantly negatively correlated with serum ALP in group 2 and with urinary calcium/creatinine ratio in group 1. Duration of TPN as short as 2 weeks can affect negatively the BMC as documented by DEXA scan in preterm infants receiving TPN without phosphorus supplementation.

Key words: Bone mineral content, osteoporosis of prematurity, preterm, total parenteral nutrition

INTRODUCTION

Osteopenia of Prematurity (OP) occurs when bone mineral content in an infant is significantly decreased compared to that seen in the fetus or infant of comparable size or gestational age. The incidence seen in very-low-birth-weight infants is approximately 30% and increases with infants born less than 28 weeks gestation (Cross and Vasquez, 2000). An inadequate supply of nutrients (vitamin D, calcium and phosphorus), a prolonged period of total parenteral nutrition, immobilisation and the intake of some drugs are the main factors involved in the pathogenesis of osteopenia (Demarini, 2005).

Neonatal osteopenia is a well-known condition that predisposes to pathological fractures in newborn infants. Mineral deficiency leading to abnormal bone formation is the commonest cause of neonatal osteopenia (Lam et al., 2007). It is important to recognize and prevent this condition as Fewtrell et al. (2000), found that the linear growth of children with neonatal metabolic bone disease was significantly reduced, even at the age of 12 years.

DEXA scan is currently the most extensively employed method for measuring BMC in pediatrics because it delivers low radiation dose and the time required for the procedure is short. DEXA scans are sensitive in detecting small changes in BMC and density and can predict risks of fractures. Its use is now validated in preterm and term infants (Syed and Khan, 2002). Reference values of body composition in preterm and term infants at birth have been reported (Rigo et al., 1998).

Hypercalciuria is a complication of both short- and long-term Total Parenteral Nutrition (TPN) and contributes to the risk of negative Ca balance and metabolic bone disease. The degree of hypercalciuria associated with TPN is known to be influenced by components of TPN formula, supplementing TPN with inorganic phosphorus reduces TPN-induced hypercalciuria (Berkelhammer et al., 1998). Failure to provide adequate calcium and phosphorus is critical in the development of nutrition-related bone disorders. Bone mineral retention correlates with calcium and phosphorus intake and the calcium-phosphorus ratio (Ferrone and Geraci, 2007).

Intravenous phosphorus preparation was not available in Egypt till recently. With its introduction in Egypt, we aimed to prove the positive effect of adding intravenous phosphorus to TPN on Ca and PO4, metabolism of preterm neonates by measuring bone mineral content using DEXA scan.
MATERIALS AND METHODS

This was a case-control study which was conducted on 30 preterm infants who were admitted to NICU of Obstetric and Gynecology Hospital at Ain Shams University from March 2008 to October 2008. The study protocol was approved by institutional ethics committees of Pediatric Hospital.

Subjects: Newborn infants born at 36 or less weeks of gestation were eligible for enrolment; if they needed total parenteral nutrition for a period not less than 4 days and started total parenteral nutrition at the 2nd day of life. Neonates were excluded from the study if their mothers had parathyroidal disease or if they received Furesmid or steroid therapy.

Intervention: Neonates were divided into 2 groups:

- **Group 1 (cases):** It included 15 preterm neonates who received TPN supplemented with phosphorus at a dose of (1.5 mmol/kg/day). The phosphorus formula available was (Na-Phosphate), each 1 cc contain 1 mmol phosphorus + 2 mmol Na, its trade name is (glycrophos anapule, 20 ml., Fresenius kabi company). The dose was 1.5 cc/kg/day (=1.5 mmol phosphorus + 3 mmol Na)

- **Group 2 (control):** It included 15 preterm neonates who received TPN without phosphorus supplementation

Both groups received Ca in the form of calcium gluconate (Its trade name is CaLcione, Memphis company for pharmaceutical and chemical industries Cairo-Egypt) in the dose of 5 cc/kg/day (45 mg of elemental Ca/kg/day) diluted in equal amount of glucose 5% and injected every 6 h over a period of 10 min.

Laboratory investigations: Blood samples were withdrawn from all neonates by venipuncture within 5-7 days of receiving TPN and urine was collected using a urine bag for the assay of Calcium in serum and urine, assay of phosphorus and alkaline phosphatase and calculation of urinary calcium/creatinine ratio is calculated as urinary calcium in mg dL⁻¹/urinary creatinine in mg dL⁻¹.

Abdominal U/S was done at the last day of receiving TPN to evaluate the incidence of nephrocalcinosis and was carried out with A LOGHA 400 proseries (General Electric) Duplex-Pulsed Doppler ultrasound machine with a 7.5 m HZ linear probe and high pass filter at 50 HZ.

DEXA scan (Dual Energy X-ray Absorptiometry): Lunar DPX MD+

It was used to measure total body bone mineral content. Type: Pencil beam, Whole Body Scannener. Regions: AP Spine, Lateral, Hip, Forearm/Hand, Ortho Femur, i.e., total body scan.

During measurement of the bone mineral content an infant was placed on the scanning table with the head of the infant at the marked start line, assuring that the position was the same for all subjects. The study was carried out with the infants sleeping without sedation. The infants were placed lying on their backs and were restrained with a cotton blanket. Each infant underwent scanning only once. To induce sleep, infants were fed a few minutes prior to the study. When image quality was poor due to movement of the infant or to other causes the neonate was re-scanned after sleeping again.

Statistics: Data was analyzed using Statistical Package for Special Science (SPSS) software computer program version 13. Quantitative data were described using Mean±SD, median (interquartile range); qualitative data were described in the form of numbers and percentages. Student t-test of two independent samples was used for comparison of normally distributed quantitative variables while Mann-Whitney test was used for non-parametric data. Chi-square test was used for comparison of qualitative variables. Wilcoxon signed rank test was used to compare follow up with initial values. Correlation between continuous variables was performed using Spearman correlation coefficient (r). The probability of error less than 0.05 was considered significant, while at 0.01 and 0.001 are highly significant.

RESULTS

The demographic and clinical characteristics of studied neonates are listed in Table 1. Group 1 included 15 preterm neonates with mean gestational age 33.3±1.49 weeks and mean birth weight 1.7±0.28 kg. Group 2 included 15 preterm neonates with mean gestational age 33.13±1.35 weeks and mean birth weight 1.54±0.31 kg. Except for the sex distribution which showed significant difference between both groups, there were no other significant differences.

Table 2 showed that there were no significant difference regarding serum Ca and phosphorus between group 1 and 2. Yet there were highly significant differences between both groups in the serum ALP and urinary Ca/creatinine ratio. The serum level of alkaline phosphatase and urinary Ca excretion increased significantly in the group who did not receive phosphorus in TPN.

There was also highly significant difference in the BMC between both groups. The group who received
Table 1: Descriptive data of group 1 and 2

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.A. (weeks) (Mean±SD)</td>
<td>31.35±3.1</td>
<td>31.35±3.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Birth wt. (kg) (Mean±SD)</td>
<td>3.3±1.49</td>
<td>3.3±1.35</td>
<td>0.71</td>
</tr>
<tr>
<td>Male/Female</td>
<td>11/4</td>
<td>5/10</td>
<td>0.028</td>
</tr>
<tr>
<td>Duration of TPN (days) (Mean±SD)</td>
<td>4.30±0.7</td>
<td>4.30±0.7</td>
<td>0.13</td>
</tr>
</tbody>
</table>

G.A: Gestational age, Wt: weight, TPN: Total parenteral nutrition, p<0.05: Significant, p>0.05: Non-significant

Table 2: Comparison between group 1 and 2 regarding serum Ca, PO₄, ALP, urinary Ca/Cr ratio and BMC

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Group 1 n = 15</th>
<th>Group 2 n = 15</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Ca (mg dl⁻¹)</td>
<td>9.850±0.971</td>
<td>10.230±0.829</td>
<td>0.251</td>
</tr>
<tr>
<td>Serum PO₄ (mg dl⁻¹)</td>
<td>4.240±1.535</td>
<td>4.793±1.951</td>
<td>0.395</td>
</tr>
<tr>
<td>Serum ALP (IU)</td>
<td>401.530±76.669</td>
<td>762.800±192.490</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Urinary Ca/Cr</td>
<td>0.538±0.336</td>
<td>4.253±2.870</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMC (g)</td>
<td>29.670±9.180</td>
<td>9.730±2.490</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Ca: Calcium, PO₄: Phosphorus, ALP: Alkaline phosphatase, Ca/Cr: Calcium to creatinine ratio, BMC: Bone mineral content, p<0.001: Highly significant, p>0.05: Non-significant

Table 3: Comparison between BMC of group 1 and 2 stratified according to TPN duration

<table>
<thead>
<tr>
<th>Time duration</th>
<th>Group 1 BMC</th>
<th>Group 2 BMC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤15 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPN duration</td>
<td>32.27±9.38</td>
<td>8.93±2.38</td>
<td>0.006</td>
</tr>
<tr>
<td>&gt;15 days</td>
<td>22.50±2.64</td>
<td>7.98±2.79</td>
<td>0.006</td>
</tr>
</tbody>
</table>

BMC: Bone mineral content, TPN: Total parenteral nutrition, p<0.001: Highly significant

Table 4: Correlation between BMC and GA, BW, serum Ca, PO₄, ALP, urinary Ca/Cr ratio and TPN duration in group 1 and 2

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Group 1 BMC</th>
<th>Group 2 BMC</th>
<th>r</th>
<th>p</th>
<th>sig</th>
<th>r</th>
<th>p</th>
<th>sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.A.</td>
<td>0.82±0.001</td>
<td></td>
<td>0.023</td>
<td>0.013</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth wt.</td>
<td>0.53±0.04</td>
<td></td>
<td>0.580</td>
<td>0.024</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ca</td>
<td>-0.133±0.638</td>
<td></td>
<td>-0.275</td>
<td>0.322</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum PO₄</td>
<td>-0.197±0.468</td>
<td></td>
<td>0.170</td>
<td>0.544</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum ALP</td>
<td>-0.401±0.138</td>
<td></td>
<td>-0.534</td>
<td>0.04</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca/Cr</td>
<td>-0.69±0.004</td>
<td></td>
<td>-0.328</td>
<td>0.232</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPN duration</td>
<td>-0.78±0.78</td>
<td></td>
<td>-0.27</td>
<td>0.14</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ca: Calcium, PO₄: Phosphorus ALP: Alkaline phosphatase, Ca/Cr: Calcium to creatinine ratio BMC: Bone mineral content

DISCUSSION

Premature infants are known to be at risk of developing metabolic bone disease (Salle et al., 1992). Earlier studies of bone mineralization used x-rays with the main purpose being to identify infants with rickets. By use of single-photon absorptiometry, it became possible to measure BMC, but only in a small part of the skeleton. With the development of DEXA, it has become possible to measure whole-body BMC with high precision and minimal radiation dose (Koo et al., 1995; Brunton et al., 1997).

In present study we aimed to use DEXA scan to measure bone mineral content in preterm neonates receiving total parenteral nutrition with and without phosphorus addition to TPN infusates as intravenous phosphorus formula was not available in Egypt till recently.

In present study, there was no significant difference between group 1 and 2 as regards the mean values of serum calcium and phosphorus. This was demonstrated by Catache and Leone (2003), in their study, they found that serum calcium and phosphorus levels are not good markers in early detection of mineral deficiency in very low birth weight infants and are small for gestational age.

In a national survey of many neonatal units in United Kingdom designed to assess babies at risk of osteopenia of prematurity, they demonstrated that, serum calcium is not a useful screening test as infants can maintain a normal calcium level at the expense of a loss of bone calcium. The level can also increase with phosphorus depletion and hypophosphatemia. Their data have also confirmed that although phosphate concentration is related to bone mineral density, it is not sensitive enough to identify infants with bone mineral deficits. The use of serum phosphate levels in combination with ALP levels can significantly increase the sensitivity of the screening and identification of infants at risk of metabolic bone disease (Harrison et al., 2008).

In present study we found that the mean value of serum alkaline phosphatase was significantly lower in...
group 1 compared to group 2. This means that adding phosphorus to parenteral nutrition for the preterm neonates decreases the bone turn over and improve bone growth which is indicated by the decrease in alkaline phosphatase. These results are in agreement with a study conducted by Prestridge et al. (1993); they found that the mean values of serum alkaline phosphatase were significantly decreased in 12 preterm VLBW neonates who received TPN with larger amounts of calcium and phosphorus compared to other group who received TPN with their standard content of calcium and phosphorus. Also we found significant negative correlation between BMC and serum alkaline phosphatase in group 2, the higher alkaline phosphatase, the lower the bone mineral content. Our results contradicted those of Ryan et al. (1992), they examined the relationship between bone mineralization, measured by single photon absorptiometry and alkaline phosphatase concentration in 71 preterm neonates. They demonstrated that, although alkaline phosphatase concentration was significantly related to bone mineral content, it could only explain a small amount of the variation in bone mineralization. Hence, concentration of alkaline phosphatase could not be used to identify those neonates with the greatest bone mineral deficits.

In present study, there was highly significant increase in urinary calcium/creatinine ratio in group 2 when compared to group 1. This means that adding phosphorus to parenteral nutrition reduces the urinary excretion of calcium and the urinary calcium/creatinine ratio. A study done by Catache and Leone (2003), emphasizes the importance of serial mineral urinary excretion screening in VLBW infants, since the presence of calcium near 4 mg/kg/day could be considered an early marker of phosphate deficiency and consequently of Metabolic Bone Disease (MBD) development.

Comparing the mean value of BMC measured in grams using DEXA scan between group 1 and 2, we found highly significant increase in the BMC of group 1 compared to group 2. Moreover this finding was demonstrated even when TPN duration was less than 15 days. This means that adding phosphorus to TPN received by preterm neonates had a positive effect on their bone mineral content and bone growth.

Furthermore, we found that there was highly significant negative correlation between BMC and urinary calcium/creatinine ratio in group 1. This means that the lower calcium excreted in urine, the higher is the bone mineral content.

Present results are in accordance with those of Berkelhammer et al. (1998), who demonstrated that increasing the inorganic phosphorus content of TPN formulas decreased TPN-induced hypercalciumia and potentially could improve calcium balance and bone mass in patients receiving long term TPN who are at risk for metabolic bone disease.

We also found significant positive correlation between BMC and gestational age and birth weight. Bone mineral content increased with increased gestational age and with increased birth weight in both groups. These results are in agreement with the study conducted by Avila-Diaz et al. (2001). In their study, they assessed Bone Mineral Content (BMC) of the whole skeleton, using DEXA scan, in preterm and full term infants and found that BMC measured at the second month is significantly positively correlated with both gestational age and birth weight.

So, we concluded that a period of TPN less than 15 days without phosphorus supplementation affects bone mineral content of preterm neocrates negatively and this was proved by DEXA scan measurement of BMC.

REFERENCES


