Mediators in Preterm Infants With Late-onset Sepsis: A Randomized Controlled Trial


ABSTRACT

Objective: To evaluate biochemical and clinical effects of 2 different doses of vitamin D supplementation in preterm infants with late-onset sepsis (LOS).

Study Design: A double blinded randomized controlled stratified trial included preterm infants with gestational age (GA) ≥28 weeks with LOS. Subjects were randomly assigned to receive 400 or 800 IU/day of vitamin D3. Serum concentrations of 25(OH)D, TNF-α, and IL-6 were measured at enrollment, 7 days after vitamin D supplementation, and at 40 weeks of postmenstrual age (PMA). Short-term outcomes and growth parameters were assessed.

Results: A total of 50 infants were enrolled, 25 in each group. Seventy-six percent of enrolled infants were vitamin D-deficient at enrollment in both groups whereas only one infant in the 400 IU and none in the 800 IU group remained deficient at 40 week’s PMA; vitamin D concentrations at 40 weeks PMA were 54.8 ± 35.1 and 67.4 ± 37.1 ng/mL, respectively, (P=0.01). None of the infants enrolled in the study had signs of vitamin D toxicity. Serum pro-inflammatory cytokines IL-6 and TNF-α concentrations decreased at 1 week and at discharge in both groups without differences between groups. The 2 groups did not differ in anthropometric measurements, duration of oxygen and respiratory support, duration of antimicrobial use, length of hospital stay, and mortality.

Conclusions: A dose of 400 IU of vitamin D was adequate to treat vitamin D deficiency in the majority of premature infants with LOS. The 2 dosing regimens did not differ in clinical or biochemical changes.

Key Words: 25(OH)D, IL-6, late-onset neonatal sepsis, preterm, TNF-α, vitamin D

What Is Known

- Vitamin D has antimicrobial and anti-inflammatory functions.
- Premature infants are recommended to receive vitamin D supplementation.

What Is New

- Preterm infants diagnosed with sepsis were vitamin D-deficient.
- The dose of 400 IU was sufficient to correct vitamin D deficiency in the majority of infants. The dose of 800 IU was adequate to correct vitamin D deficiency in all infants without reaching toxic level.
- Inflammatory cytokines decreased in both groups with vitamin D supplementation of 400 and 800 IU/day.
A few studies have examined its preventative role in sepsis; however, we are not aware of any study that examined the clinical and biochemical impact of vitamin D supplementation on infants with sepsis. Therefore, a double blinded randomized controlled pilot study was conducted on premature infants with late-onset sepsis (LOS). Its goal was to evaluate the effect of 2 different doses of vitamin D on serum concentrations of IL-6 and TNF-α, neonatal mortality and short-term morbidities. We hypothesized that in premature infants with LOS, supplementation with 800 IU of vitamin D will significantly reduce IL-6 and TNF-α concentrations compared to 400 IU.

SUBJECTS AND METHODS

Study Design
A randomized, double blind study was conducted at Mansoura University Neonatal Intensive Care Unit (NICU) during the period from September, 2014 to August, 2016. The study protocol was approved by the Institutional Review Board (IRB) at Mansoura University Faculty of Medicine. Parental consents were obtained before subjects’ enrollment. The study was registered at clinicaltrials.gov (identifier: NCT02273843) before recruitment of subjects and was conducted in compliance with the principles outlined in the Declaration of Helsinki.

Subjects
Infants were considered for the study when they fulfilled the following inclusion criteria: prematurity with gestational age >28 weeks and <37 weeks, postnatal age >72 hours, and presence of clinical and hematological signs suggestive of LOS. The scoring system contains 11 clinical and hematologic domains including skin color, capillary refill, tone, feeding intolerance, hepatomegaly, apnea, bradycardia, metabolic acidosis, thrombocytopenia, leukocytosis, and shift to left. The total score is 25 points. Infants were considered normal with a score <5, suspected sepsis with a score of 5–10, and clinically septic if the score exceeds 10. The accuracy of the sepsis scoring system has been validated (22). The scoring system is included in the NICU guidelines and has been routinely used in this institution for clinical purposes. Infants were not included in the study if they had major congenital anomalies, chromosomal anomalies, known inborn errors of metabolism, and immunodeficiency disorders.

Randomization
Infants with LOS were randomized using computer-generated stratified randomization codes. Clinicians and primary care-givers were masked to the intervention. Subjects were randomly assigned to 1 of 2 treatment arms of oral vitamin D3 supplements with 800 and 400 IU/day. Randomization was done by block design in 2 GA categories (28 0/7 to 33 6/7 weeks) and (34 0/7 to 36 6/7 weeks) to ensure equal number of these GA categories in each group. The allocation sequence was concealed by using sealed opaque envelopes that contained the serial number and the group to which a subject would be enrolled. After each parental consent, an envelope would be opened by the principle investigator and group assignment would be established. Care givers were blinded to the intervention. Subjects were randomly allocated stratified randomization codes. Clinicians and primary care-givers were blinded to the intervention. Subjects were randomly assigned to the intervention. Subjects were randomly assigned to the intervention.

Data Collection and Confidentiality
A data collection sheet was designed to report all relevant findings in history, physical examination, laboratory results, and complications during hospital course. Daily enteral and/or parenteral vitamin D intake and calcium/phosphorus intake from formula or total parenteral nutrition (TPN) were recorded and the overall intake was calculated. All data obtained during the study were de-identified and assigned a code with the code keys stored with the lead investigator.

Study Interventions
Oral vitamin D3 was administered in a single daily dose of 800 or 400 IU from the time of recruitment until discharge from the NICU. Antibiotic therapy and supportive care were continued according to managing physician who was not aware of the group assignment. Supplementation discontinued, and infant was to exit the study if necrotizing enterocolitis (NEC) or bowel perforation was diagnosed, and when an infant was kept nil per os (NPO) for at least 24 hours. Vitamin D was not discontinued when infants were made NPO <24 hours for various reasons, such as feeding intolerance, respiratory decompensation, and hemodynamic instability.

Feeding and Nutritional Practice
The unit guidelines are current and consistent with the American Academy of Pediatrics—fetus and newborn committee recommendations (23). Premature infants receive early parenteral nutrition with multivitamins. Trophic feeding starts at day of life one and advances with variable rates based on gestational age. Breast milk is used whenever available. premature cow’s milk formula is provided in the absence of maternal breast milk as donor’s breast milk banks are not accessible in Egypt.

Laboratory Assessment
The primary outcome of this study was the concentrations of TNF-α and IL-6 in the serum after 7 days of intervention and at 40 weeks PMA. Quantitative determination using enzyme-linked immunoassay (ELISA) was performed for this purpose (ELISA Kits, Koma Biotech Inc, Korea) (24). Serum concentrations of 25(OH)D was assessed at enrollment, at 7 days and at 40 weeks PMA, using Calbiotech’s 25(OH) Vitamin D ELISA KIT (Spring Valley, CA), it is a solid phase ELISA, based on the principal of competitive binding. This assay has analytical sensitivity: 2.5 ng/mL, specificity: 100%, <8% coefficient of variation for intra-assay and inter-assay variation, with a dynamic range: 1.25 to 150 ng/mL. Samples were obtained at enrollment, 7 days after daily vitamin D supplementation, and at 40 weeks PMA. Serum concentration of 25(OH)D was assessed at enrollment, at 7 days, and at 40 weeks PMA. Vitamin D status was categorized as deficient, insufficient, sufficient, and toxic when 25(OH)D values were <20, 20–30, >30, and >100 ng/mL, respectively (25). Other relevant laboratory investigations included quantitative determination of C-reactive protein (CRP); complete blood count (CBC), and cultures were obtained according to NICU sepsis guidelines. Cultures were incubated for 7 days according to manufacturer’s protocol before being labeled as negative.

Radiological Assessment
Renal ultrasonography was studied at 40 weeks’ PMA or at discharge from NICU whichever was earlier to detect any nephrocalcinosis.
Outcomes

Serum concentrations of TNF-α and IL-6 at 1 week after enrollment and at 40 weeks of PMA were the primary outcomes. Secondary outcomes included vitamin D status; measured by 25(OH)D at 1 week after enrollment, and at 40 weeks of PMA; bone mineral status measured by serum calcium, serum phosphorus, and urinary calcium/creatinine ratio; neonatal morbidities, for example, NEC, retinopathy of prematurity (ROP), disseminated intravascular coagulation (DIC) and renal dysfunction; durations of oxygen therapy, mechanical ventilation, total parental nutrition (TPN), antibiotic therapy, length of hospital stay; and neonatal mortality.

The trial had a data safety committee that monitored the progress of the study. The committee reviewed data on serum calcium, urinary calcium/creatinine ratio, renal ultrasonography, and mortality.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software (version 16; IBM Corporation, Armonk, NY). Kolmogorov-Smirnov test was done to examine the distribution of data. Parametric continuous variables were expressed as mean ± standard deviation, nonparametric continuous variables were expressed as median (interquartile range), and categorical variables were expressed as number (%). Student t-test was used to compare continuous parametric variables; Mann-Whitney U test was used to compare continuous nonparametric variables; chi-square test or Fisher exact test were used for categorical variables, when appropriate. Pearson’s correlation coefficient was used to test for association between normally distributed variables. Spearman correlation coefficient was used for the correlation between nonnormally distributed variables. A P-value of <0.05 was considered to be statistically significant.

Power Analysis and Sample Size Calculation

TNF-α concentrations in premature infants with sepsis were reported to be 76 ± 48 pg/mL (26). To detect 50% difference in TNF-α between the 2 groups, a sample size of 50 would be adequate (α = 0.05, β = 0.2, and power of 80%).

RESULTS

Fifty out of 73 screened infants were qualified based on the inclusion and exclusion criteria. The flow of infants in the study is shown in Figure Supplement, Supplemental Digital Content, http://links.lww.com/MPG/B551. Clinical sepsis was diagnosed in these 50 subjects. Positive blood culture was reported in 7 infants (28%) in the 400 IU group and 10 infants (40%) in the 800 IU group. Isolated organisms included: Klebsiella pneumoniae, coagulase-negative staphylococci, Staphylococcus aureus, and Escherichia coli in 43%, 29%, 14%, and 14%, respectively, in the 400 IU group versus 50%, 20%, 20%, and 10%, respectively, in the 800 IU group. The epidemiologic data, maternal characteristics, and average daily vitamin D intake from diet were similar in both groups; whereas the average total vitamin D daily intake (feeding along with supplementation) was significantly greater in the 800 IU group (Table Supplement, Supplemental Digital Content, http://links.lww.com/MPG/B551).

Serum proinflammatory cytokines IL-6 and TNF-α did not differ between the 2 groups at enrollment. The concentrations of IL-6 and TNF-α decreased significantly at 1 week and further decreased at 40 weeks (P < 0.05). However, IL-6 and TNF-α did not differ between the 2 groups at 1 week and at 40 weeks PMA (Table 1). The 2 groups did not differ in mortality or other clinical outcomes (Table 2). There were no significant differences between groups regarding body weight, length, and occipitofrontal circumference over 8 weeks (data not shown).

Serum 25(OH)D concentrations did not differ between the 400 and the 800 IU groups at enrollment (13.7 ± 6.6 vs 16.5 ± 7.6 ng/mL, P = 0.16) and 1 week later (30.6 ± 15.0 vs 35.6 ± 17.1 ng/mL, P = 0.27), whereas at 40 weeks of PMA, the 800 IU group had greater serum 25(OH)D concentrations (67.4 ± 37.1 vs 54.8 ± 35.1 ng/mL, P = 0.01) (Fig. 1). Increments in serum 25(OH)D concentrations from enrollment to 40 weeks PMA were greater in the 800 IU group than the 400 IU group (73.9 ± 34.9 vs 53.7 ± 35.7 ng/mL, P = 0.04). None of the infants enrolled in the study had a toxic serum concentration of 25(OH)D (>100 ng/mL). The majority of infants (76%) were vitamin D-deficient at enrollment; that was completely corrected in the 800 IU group and partially corrected in the 400 IU group at 40 weeks PMA (Fig. 2). Serum 25(OH)D concentrations at enrollment correlated significantly with serum calcium concentrations (r = 0.026; P = 0.01); whereas other parameters (gestational age; birth weight; serum phosphorus, alkaline phosphatase, IL-6, TNF-α, CRP; duration of antibiotics therapy, mechanical ventilation, oxygen therapy, and TPN) did not correlate with serum 25(OH)D concentrations at enrollment.

Serum calcium concentrations at enrollment and 1 week later did not differ between groups. However, after 2 weeks of supplementation, serum calcium concentrations were significantly greater in the 800 IU group and that effect continued at 40 weeks PMA (P = 0.02 and 0.003, respectively). There were no significant differences in serum phosphorus, serum alkaline phosphatase and urinary calcium/creatinine ratio at enrollment, and 40 weeks PMA (Table 3). None of the infants enrolled in the study developed nephrocalcinosis in renal ultrasound scans.

**TABLE 1.** Serum IL-6 and TNF-α concentrations during the study

<table>
<thead>
<tr>
<th></th>
<th>400 IU group (n = 25)</th>
<th>800 IU group (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 at enrollment, pg/mL</td>
<td>89.0 (74.3–109.5)</td>
<td>70.0 (80.4–248.1)</td>
<td>0.88</td>
</tr>
<tr>
<td>IL-6 at 1 week, pg/mL</td>
<td>25.0 (19.2–39.2)</td>
<td>26.9 (22.1–39.6)</td>
<td>0.49</td>
</tr>
<tr>
<td>IL-6 at 40 weeks’ PMA, pg/mL</td>
<td>2.2 (−0.5–18.7)</td>
<td>2.4 (1.8–4.4)</td>
<td>0.61</td>
</tr>
<tr>
<td>TNF-α at enrollment, pg/mL</td>
<td>151.4 (83.1–255.1)</td>
<td>104.0 (92.7–254.4)</td>
<td>0.54</td>
</tr>
<tr>
<td>TNF-α at 1 week, pg/mL</td>
<td>18.7 (20.8–53.0)</td>
<td>21.0 (26.5–77.1)</td>
<td>0.33</td>
</tr>
<tr>
<td>TNF-α at 40 weeks’ PMA, pg/mL</td>
<td>12 (6.8–32.5)</td>
<td>13.3 (11.7–19.9)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

PMA = postmenstrual age.

Data expressed as median (interquartile range), Mann-Whitney U test was used.
DISCUSSION

This study demonstrated that 76% of preterm infants with LOS had vitamin D deficiency. The daily dose of 400 IU was able to correct vitamin D deficiency in the majority of infants whereas the dose of 800 IU was effective for all infants. The high dose of vitamin D was well tolerated; however, it was not associated with superior anti-inflammatory or growth effect when compared with the dose of 400 IU/day.

This study showed decreased IL-6 and TNF-α following the supplementation of vitamin D in both groups. The study did not show a dose-response effect in relation to the 2 different doses used. The study cannot verify whether the decrease in cytokines was a response to vitamin D supplementation or to the concurrent use of

TABLE 2. Short-term outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>400 IU group (n = 25)</th>
<th>800 IU group (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality i</td>
<td>4 (16%)</td>
<td>2 (8%)</td>
<td>0.67</td>
</tr>
<tr>
<td>Shock-requiring inotropes i</td>
<td>9 (36%)</td>
<td>12 (48%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Disseminated intravascular coagulopathy i</td>
<td>3 (12%)</td>
<td>2 (8%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia i</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Patent ductus arteriosus i</td>
<td>5 (20%)</td>
<td>7 (28%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Necrotizing enterocolitis i</td>
<td>0</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
<td>Retinopathy of prematurity i</td>
<td>0</td>
<td>0</td>
<td>0.48</td>
</tr>
<tr>
<td>Duration of total parenteral nutrition, days i</td>
<td>8 (7–12)</td>
<td>7 (5–11)</td>
<td>0.32</td>
</tr>
<tr>
<td>Duration of oxygen therapy, days i</td>
<td>15 (5–21)</td>
<td>14 (5–21)</td>
<td>0.51</td>
</tr>
<tr>
<td>Mechanical ventilation i</td>
<td>15 (60%)</td>
<td>17 (68%)</td>
<td>0.77</td>
</tr>
<tr>
<td>Duration of mechanical ventilation, days i</td>
<td>4 (0–9)</td>
<td>5 (0–9)</td>
<td>0.81</td>
</tr>
<tr>
<td>Duration of antibiotic therapy, days</td>
<td>19.2 ± 5.3</td>
<td>17.8 ± 5.5</td>
<td>0.39</td>
</tr>
<tr>
<td>Length of hospital stay, days</td>
<td>34.3 ± 17.2</td>
<td>32.3 ± 14.7</td>
<td>0.66</td>
</tr>
</tbody>
</table>

1 Data expressed as mean ± standard deviation.
2 Median (interquartile range).
3 As number (%).

FIGURE 1. Serum 25 hydroxy vitamin D concentrations over time in both groups. The 400 IU/day group is represented by the dotted line and the 800 IU/day group is represented by the solid line.
antibiotics. Vitamin D is shown to decrease the production of pro-inflammatory cytokines, such as TNF-α and IL-6 in lipopolysaccharide (LPS) and Candida albicans-stimulated human peripheral blood mononuclear cells (27). Other in vitro studies did not find vitamin D status to influence LPS-induced plasma cytokine response (28). It is plausible to suspect that vitamin D supplementation at the time of sepsis may not influence the immune response to bacteria whereas having sufficient vitamin D before sepsis may be protective.

Vitamin D supplementation did not reduce the duration of mechanical ventilation or oxygen therapy. Previous studies suggested vitamin D to accelerate type II pneumocyte maturation and surfactant synthesis (29). Vitamin D deficiency in utero and early life was shown to alter lung development, decrease lung volume, and impair lung mechanics (30,31). However, these studies were conducted on fetal or neonatal lungs in absence of sepsis. The high-dose vitamin D supplementation did not influence growth parameters in this study, which is similar to previously reported RCT studies in healthy breastfed infants (32,33).

The majority of infants (76%) were vitamin D-deficient at enrollment. Vitamin D supplementation was given at a daily dose of either 400 or 800 IU; no placebo group was included as this would place infants at risk of vitamin D deficiency. The overall vitamin D intake including feeds was 640 (545–712) IU/day in the low-dose group and 1080 (909–1131) IU/day in the high-dose group. The overall intake of vitamin D did not exceed 1200 IU/day; a dose that was found to be safe in previous studies (34).

In both the 400 and 800 IU groups, serum 25(OH)D increased significantly at 40 weeks’ PMA. The magnitude of change in serum 25(OH)D was greater in the 800 IU compared with the 400 IU group. The majority (88%) of infants in the low-dose group were vitamin D-sufficient (serum 25(OH)D >30 ng/ml) at 40 weeks PMA. These data imply that the 400 IU/day dose recommended by the AAP may not be 100% sufficient to correct vitamin D deficiency in preterm infants with sepsis and would support the ESPGHAN recommendation of 800 IU/day (23,35).

The study used cholecalciferol rather than calcitriol. Calcitriol is not the physiologic vitamin D supplementation, but rather a drug with possible safety concerns. Preterm infants >24 weeks’ gestation are able to hydroxylate vitamin D in the liver and kidneys to produce the active form 1,25(OH)₂D₃. Providing calcitriol instead of vitamin D ignores the extrarenal paracrine vitamin D activation. In fact, patients who have chronic renal failure and require calcitriol supplementation also require vitamin D supplementation to support these extrarenal processes (36). Moreover, cholecalciferol may induce a more robust immune response relative to calcitriol (37).

The present study showed greater serum calcium in the high-dose group. However, serum phosphorus, ALP, and urinary Ca/Cr ratio did not differ between the 2 groups. None of the infants enrolled in this study had a toxic serum concentration of 25(OH)D (>100 ng/ml) or developed nephrocalcinosis.

This study carries many strengths. It is the first study to evaluate different doses of vitamin D supplementation on outcomes of preterm infants with LOS. The study is double-blinded, randomized controlled. Furthermore, the intake of vitamin D from nonstudy sources was calculated in both groups; also, the safety issue of higher dose of vitamin was addressed. Nonetheless, the study encountered some limitations. Vitamin D status was assessed using immune assays; recent studies showed the use of liquid chromatography tandem mass spectrometry to produce more accurate results and avoid overestimation of vitamin D concentrations (38). Even with ELISA that have a potential to overestimate serum concentrations of vitamin D, the dose of 800 IU was not toxic in any of the infants. That further supports the safety of 800 IU. However, the design of the present study was not equipped to support the adequacy of 400 IU dose because the level could have been overestimated and the dose, in such case, might be inadequate. Only 2 cytokines were tested; further exploration of cytokine expression and immunophenotyping with larger sample size may add value to future studies. The study did not evaluate long-term neurodevelopmental outcomes.
**CONCLUSIONS**

In this study population, the majority of premature infants with sepsis had vitamin D deficiency. The daily dose of 400 IU was able to correct vitamin D deficiency in the majority of infants whereas the dose of 800 IU was effective for all infants. The dose of 800 IU/day was not associated with increased risks of feeding intolerance or nephrocalcinosis. Further studies on the timing and duration of vitamin D supplementation in preterm infants with and without LOS are recommended. In addition, larger studies are required to confirm safety of the 800 IU in premature infants.

**REFERENCES**


**TABLE 3.** Serum calcium, phosphorous, alkaline phosphatase, and urinary calcium/creatinine ratio during the study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>400 IU group (n = 25)</th>
<th>800 IU group (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium at enrollment, mg/dL</td>
<td>8.2 ± 0.7</td>
<td>8.4 ± 0.7</td>
<td>0.27</td>
</tr>
<tr>
<td>Serum calcium at 1 week, mg/dL</td>
<td>8.4 ± 0.7</td>
<td>8.7 ± 0.8</td>
<td>0.14</td>
</tr>
<tr>
<td>Serum calcium at 2 weeks, mg/dL</td>
<td>8.3 ± 0.7</td>
<td>8.9 ± 0.7</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum calcium at 40 weeks’ PMA, mg/dL</td>
<td>8.5 ± 0.8</td>
<td>9.3 ± 1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Serum phosphorus at enrollment, mg/dL</td>
<td>5.0 ± 0.6</td>
<td>4.8 ± 1.1</td>
<td>0.26</td>
</tr>
<tr>
<td>Serum phosphorus at 1 week, mg/dL</td>
<td>5.1 ± 0.7</td>
<td>4.8 ± 0.8</td>
<td>0.09</td>
</tr>
<tr>
<td>Serum phosphorus at 2 weeks, mg/dL</td>
<td>5.2 ± 0.8</td>
<td>4.8 ± 0.6</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum phosphorus at 40 weeks’ PMA, mg/dL</td>
<td>5.1 ± 0.6</td>
<td>5.5 ± 0.8</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum alkaline phosphatase at enrollment, U/L</td>
<td>292 (276–324)</td>
<td>305 (287–344)</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum alkaline phosphatase at 40 weeks’ PMA, U/L</td>
<td>858 (799–892)</td>
<td>850 (794–897)</td>
<td>0.94</td>
</tr>
<tr>
<td>Urinary Ca/Cr ratio at enrollment</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.3</td>
<td>0.92</td>
</tr>
<tr>
<td>Urinary Ca/Cr ratio at 1 week</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>0.87</td>
</tr>
<tr>
<td>Urinary Ca/Cr ratio at 2 weeks</td>
<td>0.4 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.62</td>
</tr>
<tr>
<td>Urinary Ca/Cr ratio at 40 weeks’ PMA</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td>0.97</td>
</tr>
</tbody>
</table>

PMA = postmenstrual age.

*Data expressed as median (interquartile range), Mann-Whitney U test was used.*