Nephrolithiasis during the first 6 months of life in exclusively breastfed infants

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Abstract

Background We investigated etiology and prognosis of infantile nephrolithiasis, including whether lithogenic and anti-lithogenic content of breast milk affects its formation.

Methods Thirty infants with nephrolithiasis and 30 healthy infants exclusively breastfed for the first 6 months of life were included in this prospective cohort case-control study. At entry, age, sex, and timing of birth of patients and controls were recorded. All patients were diagnosed and followed up periodically using ultrasonography. All infants received oral vitamin D (400 units/day). Lithogenic (calcium, oxalate, uric acid, phosphate) and anti-lithogenic (citrate, magnesium) components of maternal milk, serum calcium, phosphate, magnesium, 25-hydroxy vitamin D and parathormone, as well as spot urine calcium, uric acid, cystine, oxalate, magnesium, citrate/creatinine ratio, and calcium/citrate ratio were compared.

Results Mean follow-up period was 56.1 ± 6.8 months. There was no difference concerning lithogenic and anti-lithogenic content of breast milk. Serum calcium, phosphorus, alkaline phosphatase, and 25-hydroxy vitamin D levels (49.1 ± 19 vs. 26.7 ± 4 ng/ml, $p < 0.001$) were significantly higher and parathormone level significantly lower in patients. Random urine calcium/creatinine and calcium/citrate ratios were significantly higher in patient group (0.63 ± 0.40 vs. 0.42 ± 0.10 and 0.62 ± 0.12 vs. 0.41 ± 0.25 mg/mg, respectively, $p < 0.01$). Three patients were lost to follow-up after the first year. At last follow-up, calculi disappeared in 25/27 remaining patients without interventions or therapy.

Conclusions Breast milk does not have an etiologic effect in infantile nephrolithiasis. Higher serum vitamin D levels may have roles in development of lower levels of PTH and higher levels of serum and urine calcium, leading to stone formation. The prognosis for infantile stones is excellent.

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Introduction

The etiology of stone development in infants is still unknown. Whereas in premature infants it may often be associated with furosemide therapy, in full-term infants, the etiology is less clear [1]. This issue was addressed in several studies that were either retrospective or did not include appropriate controls. Among the possible etiologies, they indicated hypercalciuria as the most frequent metabolic abnormality, as well as hyperoxaluria, urinary tract anomalies, systemic disease, medications, and urinary tract infections [2–8]. Taking into consideration the possible nutritional etiology of nephrolithiasis, we aimed to investigate whether breast milk and supplementation with vitamin D may have roles in the etiology of infantile urolithiasis. Furthermore, we wished to learn the prognosis of such urolithiasis in our patient population.

Methods

Between March 2014 and March 2016, according to the power analysis, 30 consecutive infants under the age of 6 months who at the time they developed nephrolithiasis were exclusively breastfed, and 30 healthy infants exclusively on breastfeeding within the first 6 months of life were included in this prospective cohort and case-control study. At the beginning of the study, age, sex, and timing of birth of the patients and controls were recorded.

We had two main outcome parameters in this study. First was breast milk content, and second was serum vitamin D level. A previous study assessing serum vitamin D levels in infants with urolithiasis was taken as reference to calculate power analysis in our study [7]. For a strong effect size level ($d = 0.8$), it was calculated that at least 56 participants (at least 28 participants for each group) needed to be included for our study to reach 90% power at 95% confidence level.

All patients were followed up by ultrasonography every 3 months in the first year. Intra-observer evaluation of the sonographic findings showed consistency of the measurements. In particular, twinkling artifact which differentiates microcalculi from other non-specific bright spots was used in the sonography of the kidneys. Patients who continued to have nephrolithiasis were checked twice a year, and patients in whom stones disappeared were checked once a year, and the last evaluation was carried out in December 2019. The criteria for inclusion were as follows: 1 month to 6 months age range, to have birth weight > 2500 g, solely breastfeeding, to have normal length and weight, to take oral vitamin D (400 U/day) according to the recommendation of the Ministry of Health, no use of any other drugs, no urinary tract infection during the follow-up period or before, and no systemic disease causing nephrolithiasis (such as renal tubular diseases). On ultrasonographic evaluation, infants with urinary system anomalies between the renal pelvis and bladder which may cause obstruction or stasis, such as stenosis of ureteropelvic and ureterovesical junction obstruction, and anomalies of bladder were excluded from the study.

Nephrolithiasis was diagnosed in all infants by ultrasonographic examination. The controls were healthy babies exclusively breastfeeding, age- and sex-matched with the study group. All controls had normal ultrasonographic evaluation. Assessment of maternal milk concerning lithogenic (calcium, oxalate, uric acid, phosphate) and anti-lithogenic content (citrate, magnesium) was carried out by HPLC (high-performance liquid chromatography) method in the physiology laboratory and reported in ng/ul. Maternal diet was not taken into account. In blood samples of both groups, the following investigations were conducted: sodium, potassium, chloride, urea, uric acid, creatinine, calcium, phosphate, magnesium, venous blood gases and bicarbonate, alkaline phosphatase, 25-hydroxy vitamin D, parathormone (PTH), AST, and ALT. Urine analysis

(random spot urine, three consecutive times) was performed for the evaluation of urinary solutes, both promoters (ratios of calcium/creatinine, cystine/creatinine, uric acid/creatinine, oxalate/creatinine, sodium/potassium) and inhibitors (ratios of magnesium/creatinine and citrate/creatinine), and calcium/citrate ratio in patients as compared with healthy controls [9, 10]. Estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula [11].

All infants were followed by ultrasonography (LOGIQ E9 GE, Healtance, Milwaukee, WI, USA). This study was approved by the Pamukkale University Ethics Committee (IRB number 60116787–020/59251) and supported by the Pamukkale University Research Fund (Number 2015TPF041).

The statistical analysis of all data was carried out using the Statistical Package for the Social Sciences (SPSS) version 17. Continuous variables were given as mean ± standard deviation, and categorical variables were given as numbers and percentages. When parametric test assumptions were provided, the significance test of the difference between the two means was used to compare independent group differences; when parametric test assumptions were not provided, the Mann-Whitney U test was used to compare independent group differences. Friedman test was used for dependent group comparisons. In addition, chi-square analysis was used to examine categorical variables.

Results

There was no significant difference between the study group and the controls concerning mean age and gender (3.8 ± 1 vs. 3.9 ± 1 months and 18/12 vs. 19/11 male/female ratio, respectively). The mean follow-up period was 56.1 ± 6.8 months. Symptoms and signs were detailed in all 30 patients to justify ultrasound. In total, 18/30 of the patients had complaints of restlessness. Concerning the history of nephrolithiasis in family members, there was no difference between the patient and control groups. Seventy-three percent of the patients and 37% of the controls were born in summer ($p = 0.004$). No treatment was administered to the patients, and they were monitored using only the intermittent physical and ultrasonographic examinations.

Concerning lithogenic and anti-lithogenic factors, there was no difference in the contents of breast milk between the study group and healthy infants (Table 1). Serum calcium, phosphorus, alkaline phosphatase, and 25(OH)-vitamin D levels were found to be significantly higher in the sera of the patients, while the parathormone level was found to be significantly lower (Table 2). Random urine calcium/creatinine and calcium/citrate ratios were significantly higher in the patient group, but the others (uric acid, oxalate, cystine, magnesium, and citrate) were normal (Table 3). There was no difference between patients and controls regarding eGFR, sodium, potassium, chloride, magnesium, or bicarbonate.

Table 1 Lithogenic and anti-lithogenic micronutrient contents of the breast milk of mothers of patients and control groups

Micronutrient contents of the breast milk (ng/ul)	Patient group (n = 30) Mean ± SD	Control group(n = 30) Mean ± SD	p
Calcium	12.61 ± 1.50	13.35 ± 1.70	ND
Phosphorus	7.89 ± 3.40	6.73 ± 2.50	ND
Oxalate	1.10 ± 0.30	0.99 ± 0.30	ND
Uric acid	38.91 ± 12.20	32.28 ± 11.70	ND
Magnesium	477.40 ± 246.50	546.87 ± 206.90	ND
Citrate	20.06 ± 12.50	15.30 ± 13.30	ND

ND no difference

The stones were bilateral in 13 infants and unilateral in 17 infants at presentation. Stone size was larger than 4 mm in four infants, between 2 and 4 mm in 24 infants, and smaller than 2 mm in two infants at the first examination. At sixth months of follow-up, 16 infants had no stone, 7 infants had stone size of < 2 mm, and 7 infants had calculus size of 2–4 mm. At the first year, stones had disappeared in 24 of 30 infants. Over the next years, 27 patients were followed up regularly, with 3 patients lost to follow-up after the first year. Nephrolithiasis had already disappeared in 2 of these 3 patients, and another had stone size < 2 mm at 1-year follow-up. At last follow-up, the calculi disappeared in 25 of the remaining 27 patients, and only two infants had stones (the stone size was < 2 mm in one, 2–4 mm in the other infant).

Discussion

In the present study, we found that breast milk content had no effect on the development of nephrolithiasis in infants younger than 6 months who are exclusively breast fed (Table 1). In contrast, previous studies have emphasized that formula feeding in infants may be a risk factor for nephrolithiasis [12, 13]. Campfield et al. [12] compared formula-fed infants and breast-fed infants concerning oxalate excretion, known as an important lithogenic factor, and found urinary oxalate excretion higher in formula-fed infants. Similarly, Hoppe et al. [13] found higher oxalate excretion in formula-fed infants, despite no difference between breast milk and formulas in terms of the mean oxalate content. In addition, it has been recently revealed in China that foods with added melamine-

contaminated milk powder also cause nephrolithiasis [14–17]. All these events indicate that infants can also be affected by nutritional factors in terms of developing nephrolithiasis. Breast milk is ultimately the best source of nutrition for infants. In this study, we showed that breast milk does not contribute to the formation of nephrolithiasis regarding its lithogenic and anti-lithogenic content.

Interestingly, we observed that children with nephrolithiasis had significantly higher levels of serum vitamin D than the control group. Moreover, other variables related to vitamin D (serum calcium, phosphorus, alkaline phosphatase, and PTH) showed changes that were appropriate for high vitamin D levels. The serum levels of calcium, phosphorus, and alkaline phosphatase, as well as urine calcium/creatinine and calcium/citrate ratios, increased in children with nephrolithiasis due to high serum levels of vitamin D compared to controls, while PTH decreased. It seems that a high serum level of vitamin D was responsible for this condition. Dependent upon high levels of serum vitamin D, hypercalciuria occurred in the patients, but since urinary citrate excretion was not elevated, the ratio of urinary calcium/citrate increased, and this situation facilitated stone formation. High urine calcium/citrate ratio has been shown to place patients at higher risk for stone formation [10]. However, parents of children with nephrolithiasis reported administering 400 units of vitamin D, just as did parents in the control group.

As recommended by the World Health Organization, in our study, both patients and controls received daily supplementation with vitamin D 10 mg (400 units) per day [18–20]. Taking into account that the breast milk is very poor in vitamin D [18, 21], one can assume that this was the only vitamin

Table 2 Comparison of biochemical data between infants with stone and infants without stones

Biochemical parameters in serum (normal range)	Patient group (n = 30) Mean ± SD	Control group (n = 30) Mean ± SD	p
25-Hydroxy vitamin D (20–80 ng/ml)	49.11 ± 19.20	26.79 ± 4.00	0.0001
Parathormone (15–65 pg/ml)	21.58 ± 8.70	35.84 ± 9.80	0.0001
Calcium (8.4–10.2 mg /dl)	10.53 ± 0.50	8.36 ± 0.40	0.0001
Phosphorus (3.5–6.6 mg/dl)	5.66 ± 0.50	4.8 ± 0.40	0.0001
Alkaline phosphatase (100–300 IU/L)	265.40 ± 93.60	160.93 ± 40.60	0.0001

Table 3 Urinary excretion of lithogenic and anti-lithogenic substances in patient and control groups

Spot urine substance/creatinine (normal values by age)	Patient group ($n = 30$) Mean $\pm$ SD	Control group ($n = 30$) Mean $\pm$ SD	p
Calcium/creatinine (< 0.8 mg/mg)	0.63 ± 0.40	0.42 ± 0.10	0.01
Cystine/creatinine (29–100 mg/g)	65.50 ± 30.00	64.40 ± 29.00	ND
Oxalate/creatinine (0.0–288 mg/g)	89.88 ± 59.90	90.13 ± 57.20	ND
Uric acid /creatinine (0.24–1.89 mg/mg)	0.38 ± 0.02	0.36 ± 0.03	ND
Citrate/creatinine (420–1136 mg/g)	1007.74 ± 330.20	1012.06 ± 390.20	ND
Calcium/citrate (mg/mg)	0.62 ± 0.12	0.41 ± 0.25	0.01
Magnesium/creatinine (> 0.13 mg/mg)	0.24 ± 0.10	0.26 ± 0.10	ND

ND no difference

D intake. Nevertheless, in the study group, serum 25-hydroxy vitamin D concentration was significantly higher than in controls (Table 2). One may speculate that it was a higher degree of exposure to sunlight in the study group, in which significantly more babies were born in the summer, that caused the difference. This difference in vitamin D level could be the source of the differences observed in blood and urine parameters between stone formers and controls (Tables 2 and 3). The association between vitamin D and nephrolithiasis was described previously [22]. However, since we did not measure serum vitamin D level in children who have not received supplementation with vitamin D during the summer months, further investigation into this hypothesis is required.

We did not administer any treatment to infants for nephrolithiasis, such as citrate or hydrochlorothiazide. Moreover, we did not make any changes to the vitamin D supplement they were receiving. However, we observed that nephrolithiasis disappeared in 27% of children in the third month, 53% in the sixth month, and 80% within the first year. Nephrolithiasis remained in only two patients after 4.5 years. The stones were < 4 mm and non-obstructive in those infants. The results of our study suggest that if there are no other risk factors for nephrolithiasis in infants fed only with breast milk, such as urinary anatomic or structural abnormalities, systemic diseases, or medications, it will not be a major problem for the future life of the baby.

This study has some limitations. We did not investigate the nucleotide content of breast milk, as nucleotides are the precursors of uric acid [23, 24]. However, there was no difference between groups with and without nephrolithiasis concerning uric acid excretion. No serum albumin was obtained to calculate corrected calcium, nor was serum ionized calcium concentration obtained. Another limitation of this study is that the vitamin D level could not be assessed in breast milk. Nevertheless, previous studies have reported that the vitamin D level in breast milk is very low [18, 21].

In conclusion, we observed that nephrolithiasis in babies within the first 6 months of life exclusively breastfed usually has a good prognosis as the vast majority of calculi disappear

spontaneously over time. Breast milk is not responsible for this situation concerning its lithogenic and anti-lithogenic content. On the other hand, the possible role of vitamin D supplementation in the development of kidney stones in infants should be further investigated.

Author's contributors Dr. Yılmaz conceptualized and designed the study, drafted the initial manuscript, designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

Prof Yüksel conceptualized and designed the study, drafted the initial manuscript, designed the data collection instruments, critically reviewed the manuscript for important intellectual content, and revised the manuscript.

Dr. Altıntaş and Prof Koçyiğit designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript.

All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Compliance with ethical standards

This study was approved by the Pamukkale University Ethics Committee (IRB number 60116787–020/59251).

Conflict of interest The authors declare that they have no conflict of interest.

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