IJKD KIDNEY DISEASES

# Elevated Serum Levels of Vitamin D in Infants With Urolithiasis

Mohammad Hossein Fallahzadeh, Javad Zare, Ghamar Hosseini Al-Hashemi, Ali Derakhshan, Mitra Basiratnia, Mohammad Mehdi Arasteh, Mohammad Amin Fallahzadeh, Mohammad Kazem Fallahzadeh

Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

**Keywords.** urinary calculi, infant, vitamin D, hypercalciuria, hypercalcemia

Original Paper

**Introduction.** The pathophysiology of urolithiasis in infancy is not well known. The aim of this study was to investigate whether infants with urolithiasis have higher serum levels of vitamin D, as a possible risk factor for urolithiasis, compared to infants without urinary calculi.

**Materials and Methods.** In this case-control study, 36 infants with urolithiasis (age range, 2.5 to 24 months) were enrolled as well as 36 age- and sex-matched infants without urolithiasis. Random urine samples were tested for calcium, phosphorous, oxalate, citrate, uric acid, sodium, potassium, magnesium, and creatinine levels, and also nitroprusside test was done on the samples. Serum levels of potassium, urea nitrogen, creatinine, 25-hydroxyvitamin D3, parathyroid hormone, calcium, phosphorous, and uric acid were measured in all of the infants with urolithiasis. Serum levels of 25-hydroxyvitamin D3 were also measured in the control group.

**Results.** Serum levels of 25-hydroxyvitamin D3 were significantly higher in the infants with urolithiasis than in the controls (33.85 ± 14.78 ng/mL versus 18.26 ± 7.43 ng/mL, $P < .001$). Nine infants in the urolithiasis group (25%) were found to have hypercalcemia; 3 of these cases also had hypervitaminosis D. Hypercalciuria was detected in 10 infants with urolithiasis (27.8%), hypocitraturia in 6 (16.7%), hypomagnesiuria in 3 (8.3%), and hyperoxaluria in 1 (2.8%). Nineteen infants with urolithiasis had at least one metabolic disorder.

**Conclusions.** High serum levels of vitamin D may play an important role in the pathogenesis of urolithiasis in infants with hypercalcemia. We recommend evaluation of vitamin D levels in these infants.

IJKD 2012;6:186-91
www.ijkd.org



## INTRODUCTION

The true incidence of urolithiasis in infancy is most probably higher than what has been previously reported. Its frequency is increasing in all age groups, including the infants. This is even true for urolithiasis in nonendemic regions.[1-4] Reports on the geographic variation in the prevalence of urolithiasis in the United States show a 50% higher prevalence in the Southeast (the "kidney stone belt") as compared with the Northwest.[5] Higher frequencies may be related to many factors including better selection of the patients for renal ultrasonography even with nonspecific urinary tract symptoms. Risk factors for urolithiasis include genetic, metabolic, infectious, nutritional, environmental, anatomical, and drug-related disorders. In addition, metabolic patterns of urolithiasis may change over time.[5,6] A significant number of the patients may have more

than one risk factor, and on the other hand, no cause can be found in a few patients.[7] Therefore, full investigation for all the possible risk factors of urolithiasis is crucial.

Due to routine prophylactic use of vitamin D in infants and possibly satisfactory sunlight exposure in this geographic area, and also the role of vitamin D in metabolism of calcium, this study was carried out to find the possible role of higher serum levels of vitamin D as a risk factor for kidney calculus formation during infancy.

## MATERIALS AND METHODS

In this case-control study, 36 infants (aged 2.5 to 24 months) with newly diagnosed urolithiasis were recruited. All the infants were visited by pediatric nephrologists in the Pediatric Nephrology Clinic of Shiraz University of Medical Sciences, from October 2010 to April 2011. The exclusion criteria for enrollment were histories of prematurity, low birth weight (< 2.5 kg), neonatal intensive care unit admission, recent prolonged hospital admission, and consumption of steroids, diuretics, calcium supplementations, and other medications that can promote calculus formation. In addition, 36 age- and sex-matched infants referred for routine checkup were enrolled as controls. The exclusion criteria for enrollment of controls were prematurity, low birth weight (< 2.5 kg), kidney diseases, kidney calculi or the related symptoms, chronic diseases, and failure to thrive.

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences and was done in accordance with the Declaration of Helsinki. Written consents were obtained from all the parents. Complete history taking and physical examination were performed. All the patients had at least 2 ultrasonography reports, and the last ultrasonography was performed by a radiology expert in the pediatric field with a curved probe of 7.5 MHz for larger infants and a 3.5-MHz probe was used for smaller infants. The ultrasonography device was the LOGIQ 9 (GE Healthcare, USA). The ultrasonographic criteria for diagnosis of urolithiasis were presence of a hyperechoic lesion with a posterior acoustic shadow in the pyelocaliceal system, ureter, or urinary bladder.

For all enrolled infants, a complete metabolic workup was done. Blood and urine samples of all infants were immediately transferred to the laboratory for analysis on the same day. Urine investigations included urinalysis, urine culture, nitroprusside test, and measurement of sodium, potassium, calcium, uric acid, magnesium, oxalate, citrate, and creatinine in random urine. Furthermore, serum levels of calcium, phosphorous, alkaline phosphatase, potassium, sodium, uric acid, urea nitrogen, creatinine, 25-hydroxyvitamin D3 (vitamin D), and parathyroid hormone were measured in all cases. Moreover, venous blood gas analyses were performed for all cases. Additionally, serum levels of vitamin D were measured in the controls. Urinary levels of calcium and magnesium and serum levels of calcium and alkaline phosphatase were measured by photometric method. Urinary levels of oxalate and citrate levels were measured by the high-performance liquid chromatography method. Serum levels of vitamin D and parathyroid hormone were quantified by electrochemiluminescence and immunochemiluminescence methods, respectively (Diasorin Kits, Italy). Reference ranges were 7.35 to 7.45 for serum pH, 20 mEq/L to 28 mEq/L for bicarbonate, 35 mm Hg to 45 mm Hg carbon dioxide partial pressure, and -2 to 2 for base excess. Definitions of normal serum and urinary values of the measured parameters are shown in Table 1.

**Table 1.** Reference Ranges of Measured Parameters in Infants

| Parameter | Reference Range |
|---|---|
| Urine calcium/creatinine, mg/mg | |
| < 6 months | < 0.8 |
| 6 to 12 months | < 0.6 |
| 13 to 24 months | < 0.52 |
| Urine oxalate/creatinine, mg/mg | |
| 1 to 6 months | < 0.288 |
| 6 to 24 months | < 0.139 |
| Urine magnesium/creatinine, mg/mg | |
| 1 to 12 months | 0.084 to 0.484 |
| 13 to 24 months | 0.084 to 0.358 |
| Urine citrate/creatinine, mg/mg | |
| 1 to 24 months | > 0.2 |
| Uric acid clearance, mg/dL GFR | |
| 1 to 24 months | < 3.3 |
| Urine phosphate/creatinine, mg/mg | |
| 1 to 12 months | 0.33 to 5.22 |
| 13 to 24 months | 0.33 to 3.85 |
| Serum parathyroid hormone, pg/mL | |
| 1 to 24 months | 10 to 80 |
| Serum vitamin D, ng/mL | |
| 1 to 24 months | 10 to 55 |
| Serum Phosphorus, mg/dL | |
| < 5 months | 4.8 to 7.4 |
| 5 to 24 months | 4.5 to 6.2 |

Pyuria and hematuria were defined as more than 5 leukocytes and erythrocytes per high-power field, respectively.

Statistical analyses were done using the SPSS software (Statistical Package for the Social Sciences, version 15.0, SPSS Inc, Chicago, Ill, USA). The independent *t* test was used to compare continuous data with normal distribution and the Mann-Whitney U test was used to compare continuous data with skewed distribution between the two groups. The chi-square and Fisher exact tests were used for comparison of categorical data between the two groups. Correlations of continuous data with normal distribution were tested using the Pearson correlation coefficient. Moreover, correlations of continuous data with abnormal distribution were tested using the Spearman correlation coefficient. *P* values less than .05 were considered significant.

## RESULTS

The infants with urolithiasis (24 boys and 12 girls) were 2.5 to 24 months old with a mean age of 8.4 ± 4.7 months (median age, 7.25 months). The mean age of the control infants (22 boys and 14 girls) was 8.7 ± 4.7 months (median age, 8.5 months; range, 2 to 24 months). There were no significant differences between the two groups in terms of age and gender ($P = .59$ and $P = .62$, respectively). All of the participant infants had normal heights and weights for age. History of kidney calculus was positive in 80.6% of the first- or second-degree relatives of the infants with urolithiasis.

All of the participants had started on oral vitamin D (400 IU/d) since the neonatal period as the routine preventive program recommended by the Ministry of Health. However, 13 infants with urolithiasis and 17 controls were not taking vitamin D regularly ($P = .34$). The mean duration of direct sunlight exposure, reported by the parents, was 16.53 ± 24.97 min/d in the urolithiasis group and 12.06 ± 12.16 min/d in the control group ($P = .90$). Thirty infants with urolithiasis were breastfed.

Measured values of urinary and serum parameters are shown in Table 2. Serum levels of sodium, potassium, uric acid, and creatinine were within reference ranges in all of the infants. None of the infants with urolithiasis had metabolic acidosis or alkalosis; serum bicarbonate as well as blood pH and carbon dioxide partial pressure were within reference ranges. Urinary sodium-potassium ratios were more than 2.5 in 4 patients. No cystine crystals were found and nitroprusside test was negative in all the cases. Hypercalciuria was detected in 10 infants with urolithiasis (27.8%), hypocitraturia in 6 (16.7%), hypomagnesiuria in 3 (8.3%), and hyperoxaluria in 1 (2.8%). None of them had hyperuricosuria. Pyuria and hematuria were reported in 6 (16.7%) and 7 (19.0%) of these infants, respectively. All of the infants with urolithiasis had negative urine cultures at the time of this study, but a history of previous urinary tract infection was present in 6.

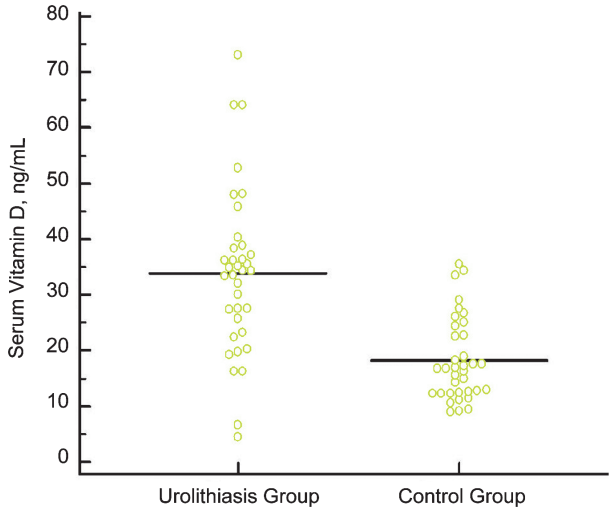
Serum levels of vitamin D were high in 3 and low in 2 of the patients in the urolithiasis group and normal or low in all of the controls (Table 3). The mean serum levels of vitamin D was significantly higher in the urolithiasis group compared to the controls (33.58 ± 14.77 ng/mL versus 18.26 ± 7.42 ng/mL, $P < .001$; Figure). In the urolithiasis group, serum levels of vitamin D had

**Table 2.** Laboratory Studies

| Parameter | Median (Range) |
|---|---|
| Urine calcium/creatinine, mg/mg | 0.34 (0.02 to 1.23) |
| Urine oxalate/creatinine, mg/mg | 0.04 (0.01 to 0.6) |
| Urine magnesium/creatinine, mg/mg | 0.15 (0.07 to 0.45) |
| Urine citrate/creatinine, mg/mg | 0.73 (0.05 to 5.89) |
| Uric acid clearance, mg/dL GFR | 0.93 (0.4 to 1.8) |
| Serum calcium, mg/dL | 10.7 (9.2 to 12.5) |
| Serum parathyroid hormone, pg/mL | 44.55 (13.2 to 158) |
| Serum vitamin D, ng/mL | 34.31 (4.5 to 73.1) |
| Serum phosphorus, mg/dL | 6.0 (4.5 to 10.5) |

**Table 3.** Characteristics of Patients With Abnormal Serum Levels of Vitamin D

| Patient | Age, mo | Vitamin D Level, ng/mL | Serum Calcium, mg/dL | Serum Posphorus, mg/dL | Urine Calcium/ Creatinine | Parathyroid Hormone, pg/mL | Serum Alkaline Phosphatase, U/L |
|---|---|---|---|---|---|---|---|
| 1 | 9 | 64.0 | 12.0 | 4.5 | 0.60 | 51.1 | 231 |
| 2 | 6 | 64.0 | 12.5 | 10.5 | 0.60 | 28.7 | 499 |
| 3 | 4 | 73.1 | 10.9 | 6.2 | 0.15 | 44.1 | 853 |
| 4 | 5 | 44.8 | 12.1 | 5.6 | 0.25 | 99.4 | 542 |
| 5 | 11 | 6.7 | 9.2 | 5.5 | 0.02 | 158.0 | 254 |




Serum levels of vitamin D (ng/mL) in infants with and without urolithiasis ($P < .001$).

a statistically significant correlation with serum levels of calcium (r = 0.42, $P = .01$). However, no significant correlations were found between serum levels of vitamin D and urinary calcium-creatinine ratios or serum levels of parathyroid hormone. Serum levels of calcium were significantly higher in the urolithiasis group with hypercalciuria compared to normocalciuric ones (11.06 ± 0.81 mg/dL versus 10.61 ± 0.61 mg/dL, $P = .02$). Although hypercalciuric patients had higher serum levels of vitamin D and lower serum levels of parathyroid hormone compared to normocalciuric ones, these differences did not reach statistical significance.

No anatomical abnormalities of the kidneys or urinary tract were detected by ultrasonography evaluation except for a low-grade vesicoureteral reflux in 1 infant with urolithiasis. The cause of performing the first ultrasonography was irritability (41.7%), urinary difficulty or dribbling (19.4%), previous urinary tract infection (11.1%), gross hematuria (8.3%), vomiting (5.6%), passage of urinary calculi (5.6%), and other symptoms (8.4%). The calculi were bilateral (38.9%), in the left kidney (36.1%), and in the right kidney (19.4%). Two patients had ureteral calculi in addition to the kidney calculus. No bladder calculi were detected. The calculi were 3 mm in diameter or smaller in 16 infants (44.4%), larger than 3 mm but smaller than 5 mm in 9 (25%), 5 mm to 10 mm in 6 (16.7%), and greater than 10 mm in 5 (13.9%) patients. In 30 patients, 2 calculi or more were detected.

## DISCUSSION

In this case-control study, we demonstrated for the first time that infants with urolithiasis had higher serum levels of 25-hydroxyvitamin D3 compared to healthy controls. Infantile urolithiasis has been addressed in several previous studies from different parts of the world.[4,8-13] However, few of those reports focused on infantile urolithiasis as a separate entity. In addition, most previous reports were retrospective. There are many reports on the role of vitamin D receptor gene in the patients with urolithiasis with conflicting results. In a recent meta-analysis, it was concluded that vitamin D receptor polymorphisms could be potential biomarkers for urolithiasis susceptibility.[14] However, in a case-control study that evaluated the role of vitamin D in adults with urinary calculi, it was found that the main vitamin D metabolites have no pathophysiologic role in urinary calcium calculus formation.[15]

With thorough evaluation, predisposing factors are expected to be found in more than 75% of the children with urolithiasis.[16,17] The most common metabolic abnormalities reported in children with urolithiasis are hypercalciuria, hypocitraturia, and hyperoxaluria.[18-24] In a recent report from our center, which evaluated both infants and children with urinary calculi, the most common metabolic abnormalities were hypomagnesiuria and hypocitraturia.[25] Like other reports, in the current study, hypercalciuria and hypocitraturia were among the most common metabolic abnormalities in infants with urolithiasis; however, hyperoxaluria was only found in 1 patient.

Although most cases of hypercaluria are idiopathic, in some patients it can be secondary to hypercalcemia. Other causes of secondary hypercalciuria include hyperparathyroidism, bone resorption due to different etiologies, and vitamin D toxicity.[26] Although they are less likely to cause vitamin D toxicity, excessive sunlight exposure and routine prophylactic vitamin D administration to infants may lead to high serum levels of vitamin D. In our study, serum levels of vitamin D were significantly higher in the infants with urolithiasis than in the controls. Furthermore, although not significantly, infants with urolithiasis received more sunlight exposure and higher numbers of them were consuming regular prophylactic vitamin D therapy compared to the healthy controls. Therefore, more

sunlight exposure and routine prophylactic vitamin D therapy may have contributed to higher serum levels of vitamin D in the infants of our study.

In the current study, a significant positive correlation was found between serum levels of vitamin D and calcium. Moreover, hypercalciuric cases had significantly higher serum levels of calcium than normocalciuric cases. Hypercalciuric cases also had higher serum levels of vitamin D and parathyroid hormone; however, these differences were not significant. Additionally, of the three cases with hypervitaminosis D, 2 had hypercalcemia and hypercalciuria. Therefore, it appears that high serum levels of vitamin D, by leading to hypercalcemia and then hypercalciuria, may contribute to formation of urolithiasis in some infants. Although a significant correlation between urinary calcium-creatinine ratio and serum levels of vitamin D was not found in our study, this correlation may reach significance in future studies with larger sample sizes.

As it was reported from Turkey as well,[6] the most frequent symptom of urolithiasis was irritability in our study, but incidental finding of urolithiasis was not observed. The locations of the calculi in our study were in accordance with the report from Western Turkey,[19] but the rate of bilateral and multiple calculi was lower in Turkish infants than the participants in the present study and our previous report.[27] In the current study, no bladder calculi were detected. Furthermore, no ureteral calculi independent on kidney calculi were found.

## CONCLUSIONS

We can conclude that elevated serum levels of vitamin D may play a role in the pathogenesis of infantile urolithiasis, particularly in the patients with hypercalcemia. Further studies with larger sample sizes are needed to better clarify the role of high serum levels of vitamin D in infantile calculi. Furthermore, these findings should be interpreted with caution because of the limitations of this study, including not extending the study to all seasons with different amounts of sunlight exposure, relatively low sample size, and not performing complete metabolic evaluation of controls.

## FINANCIAL DISCLOSURE

This study was financially supported by a grant (No 5364) from Shiraz University of Medical Sciences as a contribution to a subspecialty thesis of Dr Javad Zare in 2010.

## CONFLICT OF INTEREST

None declared.

## REFERENCES

1. VanDervoort K, Wiesen J, Frank R, et al. Urolithiasis in pediatric patients: a single center study of incidence, clinical presentation and outcome. J Urol. 2007;177: 2300-5.
2. Stamatelou KK, Francis ME, Jones CA, Nyberg LM, Curhan GC. Time trends in reported prevalence of kidney stones in the United States: 1976-1994. Kidney Int. 2003;63:1817-23.
3. Lopez M, Hoppe B. History, epidemiology and regional diversities of urolithiasis. Pediatr Nephrol. 2010;25:49-59.
4. Sarkissian A, Babloyan A, Arikyants N, Hesse A, Blau N, Leumann E. Pediatric urolithiasis in Armenia: a study of 198 patients observed from 1991 to 1999. Pediatr Nephrol. 2001;16:728-32.
5. Soucie JM, Coates RJ, McClellan W, Austin H, Thun M. Relation between geographic variability in kidney stones prevalence and risk factors for stones. Am J Epidemiol. 1996;143:487-95.
6. Guven AG, Koyun M, Baysal YE, et al. Urolithiasis in the first year of life. Pediatr Nephrol. 2010;25:129-34.
7. Milliner DS, Murphy ME. Urolithiasis in pediatric patients. Mayo Clin Proc. 1993;68:241-8.
8. Edvardsson V, Elidottir H, Indridason OS, Palsson R. High incidence of kidney stones in Icelandic children. Pediatr Nephrol. 2005;20:940-4.
9. Ozokutan BH, Kucukaydin M, Gunduz Z, Kabaklioglu M, Okur H, Turan C. Urolithiasis in childhood. Pediatr Surg Int. 2000;16:60-3.
10. Coward RJ, Peters CJ, Duffy PG, et al. Epidemiology of paediatric renal stone disease in the UK. Arch Dis Child. 2003;88:962-5.
11. Safarinejad MR. Urinary mineral excretion in healthy Iranian children. Pediatr Nephrol. 2003;18:140-4.
12. Ali SH, Rifat UN. Etiological and clinical patterns of childhood urolithiasis in Iraq. Pediatr Nephrol. 2005;20:1453-7.
13. Ammenti A, Neri E, Agistri R, Beseghi U, Bacchini E. Idiopathic hypercalciuria in infants with renal stones. Pediatr Nephrol. 2006;21:1901-3.
14. Lin Y, Mao Q, Zheng X, Chen H, Yang K, Xie L. Vitamin D receptor genetic polymorphisms and the risk of urolithiasis: a meta-analysis. Urol Int. 2011;86:249-55.
15. Netelenbos JC, Jongen MJ, van der Vijgh WJ, Lips P, van Ginkel FC. Vitamin D status in urinary calcium stone formation. Arch Intern Med. 1985;145:681-4.
16. Sarica K. Pediatric urolithiasis: etiology, specific pathogenesis and medical treatment. Urol Res. 2006;34:96-101.
17. van't Hoff WG. Aetiological factors in paediatric

urolithiasis. Nephron Clin Pract. 2004;98:c45-8.

18. Tabel Y, Akin IM, Tekin S. Clinical and demographic characteristics of children with urolithiasis: single-center experience from eastern Turkey. Urol Int. 2009;83:217-21.
19. Ertan P, Tekin G, Oger N, Alkan S, Horasan GD. Metabolic and demographic characteristics of children with urolithiasis in Western Turkey. Urol Res. 2011;39:105-10.
20. Naseri M, Varasteh AR, Alamdaran SA. Metabolic factors associated with urinary calculi in children. Iran J Kidney Dis. 2010;4:32-8.
21. Sepahi MA, Heidari A, Shajari A. Clinical manifestations and etiology of renal stones in children less than 14 years age. Saudi J Kidney Dis Transpl. 2010;21:181-4.
22. Gurgoze MK, Sari MY. Results of medical treatment and metabolic risk factors in children with urolithiasis. Pediatr Nephrol. 2011;26:933-7.
23. Fallahzadeh MK, Fallahzadeh MH, Mowla A, Derakhshan A. Hypercalciuria in children with urinary tract symptoms. Saudi J Kidney Dis Transpl. 2010;21:673-7.
24. Safaei Asl A, Maleknejad S. Pediatric urolithiasis: an experience of a single center. Iran J Kidney Dis. 2011;5:309-13.
25. Fallahzadeh MH, Fallahzadeh MK, Sedighi V, et al. A report on metabolic evaluation of 153 children with urolithiasis. Pediatr Nephrol. 2010;25:1866-7.
26. Pont A. Unusual causes of hypercalcemia. Endocrinol Metab Clin North Am. 1989;18:753-64.
27. Fallahzadeh MH, Sedighi V, Fallahzadeh MK, Fallahzadeh MA, Derakhshan A. Number, location and size of the stones in 153 children with urolithiasis. The 15th Congress of International Pediatric Nephrology Association. 29 August- 2 September, 2010, New York, USA.

Correspondence to:
Mohammad Kazem Fallahzadeh, MD
Department of Pediatrics, Nemazee Hospital, Shiraz, Iran
E-mail: fallahzadeh@gmail.com

Received October 2011
Revised January 2012
Accepted January 2012