

# THE ROLE OF ACTIVE VITAMIN D ON STONE FORMATION AND HYPERCALCIURIA

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## ABSTRACT

**Objective:** Urolithiasis is a multifactorial disease in which both genetic and environmental factors have effects on onset and severity of disease. In this study, the levels of serum 1,25(OH)<sub>2</sub>D<sub>3</sub> in children with urolithiasis and hypercalciuria and the factors affecting it were investigated.

**Material and Method:** A group of forty three children (mean age 8.88±4.62 years, 49% male) diagnosed with urolithiasis and hypercalciuria were included in the study. The study group who had stones in ultrasonography was classified as group 1 and group who had only hypercalciuria, but no stone in ultrasonography was classified as group 2.

Twenty three healthy children constituted the control group. In all cases, the serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels were investigated.

**Results:** 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in group 1 and 2 were significantly higher than the control group. Only 24-hour urine Ca excretion (p=0.000) and 24-hour urine volume (p=0.001) were higher in group 2 than group 1.

**Conclusion:** Increase in active vitamin D might have a role in the pathogenesis of urolithiasis and hypercalciuria.

**Key Words:** Urolithiasis, 1,25(OH)<sub>2</sub>D<sub>3</sub>, children, calcium, phosphorus *Nobel Med 2013; 9(3): 88-91*

## TAŞ OLUŞUMU VE HİPERKALSİÜRİDE AKTİF D VİTAMİNİNİN ROLÜ

### ÖZET

**Amaç:** Ürolitiazis multifaktöryel hastalık olup, genetik ve çevresel faktörler hastalığın başlangıcı ve şiddetini etkiler. Bu çalışmada ürolitiazisli ve hiperkalsiürlü çocukların serum 1,25(OH)<sub>2</sub>D<sub>3</sub> düzeyleri ve onu etkileyen faktörler araştırılmıştır.

**Materyal ve Metod:** Ürolitiazis ve hiperkalsiüri olan 43 çocuk (ortalama yaş 8,88±4,62 yıl, %49 erkek) çalışmaya dahil edildi. Ultrasonografide (USG) taşı olanlar 1. grup, sadece hiperkalsiüri olup USG'de taşı

olmayanlar 2. grup olarak ayarlandı. 23 sağlıklı çocuk kontrol grubunu oluşturdu. Tüm çocukların serum 1,25(OH)<sub>2</sub>D<sub>3</sub> düzeyleri araştırıldı.

**Bulgular:** 1. ve 2. grubun serum 1,25(OH)<sub>2</sub>D<sub>3</sub> düzeyleri kontrol grubundan anlamlı olarak yüksek saptandı. 2. grubun 24 saatlik idrar kalsiyum atılımı (p=0,000) ve 24 saatlik idrar volumü (p=0,001) 1. gruptan yüksek bulundu.

**Sonuç:** Ürolitiazis ve hiperkalsiüri patogenezinde aktif D vitamini rol alıyor olabilir.

**Anahtar Kelimeler:** Ürolitiazis, 1,25(OH)<sub>2</sub>D<sub>3</sub>, çocuklar, kalsiyum, fosfor *Nobel Med 2013; 9(3): 88-91*

## INTRODUCTION

Urolithiasis is crystallized mass involved in protein and lipids of the kidney or urinary tract and may occur due to metabolic, endocrinological and urological causes.<sup>1</sup> Hypercalciuria is one of the metabolic disorders causing urolithiasis.<sup>2</sup> Urolithiasis is an important disorder that

can be seen in all childhood period.<sup>3</sup> Genetic and environmental factors determine the morbidity and severity of urolithiasis which is a multifactorial disease.<sup>4</sup>

Turkey is a country in which pediatric urinary tract stone disease is seen endemically and which 17% of the total population have stone disease.<sup>5</sup> →

Vitamin D, a secosteroid structure, is the most important hormone in the regulation of intracellular and extracellular Ca and phosphorus. 1,25(OH)<sub>2</sub>D<sub>3</sub> accelerates selectively gene transcription on target cell nucleus of steroid-receptor complex by connecting to intracellular vitamin D receptor (VDR) in all tissues. The genetic defect of VDR increases the risk of stone formation.<sup>6</sup>

In this study, the aim is to investigate the levels of serum 1,25(OH)<sub>2</sub>D<sub>3</sub> in children with urolithiasis and hypercalciuria and the factors affecting it.

## MATERIAL and METHOD

Forty three children (mean age 8.88±4.62 years, min-max 1-16 years, 49% male) with the diagnosis of urolithiasis and hypercalciuria were included in the study. The study was conducted during January-February 2010 (in winter) in order to minimize the effect on the levels of seasonal vitamin D on the levels. In accordance with Helsinki Declaration, the study was performed by receiving the signed consent of the families of the children. The study group who had stones in ultrasonography (female/male=13/12, mean age±SD 8.08±5.15 years) was classified as group 1 and group who had only hypercalciuria but no stone in ultrasonography (female/male=9/9, mean age±SD 10.8±3.14 years) was classified as group 2. Stones of all size have distinguishing characteristics of echogenicity and shadowing on ultrasonography. All patients in group 2 were performed an ultrasonographic examination to rule out stones. Patients with a history of infection and vitamin use were excluded. Patients with urolithiasis and hypercalciuria were evaluated for geographic variation. Twenty three healthy children with similar age and gender constituted the control group (group 3). Neither the children in the control group nor their first and second degree relatives had history of stone. Hypercalciuria was defined as 24-hour urinary calcium excretion in the urine >4 mg/kg/d or spot calcium/creatinine ratio >0.21 (mg/mg). As the value of urine calcium/creatinine greater than 0.2 as an indicator of hypercalciuria can be used only in children older than 6 years. We used the normally Ca/Cr ratio for age in younger children. Further 24-hour urine Ca was obtained in all children in group 2, but were not obtained from 7 children in group 1 because of their young age. Stone analysis was performed on eight children in group 1. Also spot Ca/Cr results were available for all children in group 1 and 2. Smaller, nonobstructing stones were managed medically. The patients with urolithiasis were prescribed with sodium limited diet and high daily fluid intakes which are the most valuable therapeutic options. Serum Ca, phosphorus (P), parathyroid

**Table 1:** Demographic data of the study group

	Gender		Origin		History of Stone	
	f (%)	m (%)	West (%)	East (%)	Yes (%)	No (%)
Group 1 (n=25)	52	48	52	48	80	20
Group 2 (n=18)	50	50	44	56	44	56
Group 3 (n=23)	47.8	52.2	54	46	0	100

Group 1: Hypercalciuria with urolithiasis, Group 2: Hypercalciuria without urolithiasis, Group 3: Control

**Table 2:** The comparisons between group 1, 2 and group 3

	Group 1 (n=25)	Group 2 (n=18)	Group 3 (n=23)	p value
Age (year)	8.08±5.18	10.6±3.14	10.2±3.64	NS <sup>a</sup>
Female/Male (n)	13/12	9/9	11/12	NS <sup>a</sup>
Serum Ca (mg/dl)	9.71±0.15	9.61±0.55	9.51±0.14	NS <sup>a</sup>
Serum P (mg/dl)	4.58±0.69	4.43±0.67	4.23±0.43	NS <sup>a</sup>
iPTH (pg/ml)	52.24±49.59	53.88±19.74	50.88±10.74	NS <sup>a</sup>
Urine Ca/Cr (mg/mg)	0.17±0.12	0.25±0.18	0.18±0.05	NS <sup>a</sup>
24-hours Urine Ca (mg/kg/d)	2.55±0.95	5.48±2.29	2.78±0.08	0.000 <sup>a</sup>
24-hours urine volume (ml)	777.7±321.2	1086.7±189	900.7±28.2	0.000 <sup>a</sup>
1,25(OH) <sub>2</sub> D <sub>3</sub> (pg/ml)	100.81±38.59	113.43±34.75	63.84±18.80	0.000 <sup>a</sup>

Group 1: Hypercalciuria with urolithiasis, Group 2: Hypercalciuria without urolithiasis, Group 3: Control, NS: None significant, a: p value for Group 1, 2 and 3

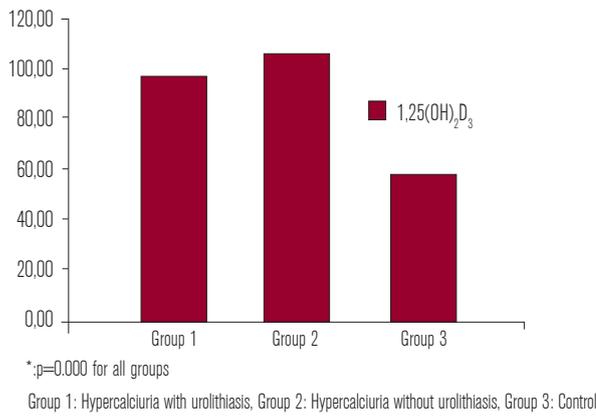
hormone (PTH), alkaline phosphatase (ALP), blood gases, 24-hour or spot urine calcium excretion, 24-hour urine oxalate, citrate, cystine excretion and urolithiasis analysis and ultrasonography (USG) were studied. In all cases, the serum 1,25(OH)<sub>2</sub>D<sub>3</sub> levels (Immundiagnostic AG Stubenwald-Allee 8A, 64625 Benshei, GERMANY) were investigated by ELISA method in the company's catalog number K2112.

Results of p value lower than 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS 15.0. Multiple linear regression analysis, ANOVA and t-test were used.

## RESULTS

There was no significant difference in gender, age and origin between group 1 and group 2 (p>0.05). The first symptoms of the cases at the administration to our clinic were most commonly stone (58%), hematuria (28%), pain (7%) and dysuria (7%). Although family history of urinary stones or hypercalciuria in the study group were detected as 65% positive, the history of urinary stone was found to be significantly more frequent in group 1 (80%) than in group 2 (44%) (p<0.05) (Table 1).

1,25(OH)<sub>2</sub>D<sub>3</sub> levels of the cases with stone (group 1) (100.81±38.59 pg/ml) and without (group 2) (113.43±34.75 pg/ml) were separately and →



**Figure 1:** The comparison of 1,25(OH)<sub>2</sub>D<sub>3</sub> in all groups

significantly higher than the control group ( $p=0.000$ ), but there was no significant difference between 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in group 1 and group 2 ( $p=0.277$ ) (Figure 1). Only 24-hour urine Ca excretion ( $p=0.000$ ) and 24-hour urine volume ( $p=0.000$ ) were different in all groups. There was no difference between other measurements including PTH ( $p>0.05$ ) (Table 2).

In nine of 25 children with urolithiasis, 24 hr urine analysis was performed. Mostly hyperoxaluria (55.2%) was detected and it was respectively followed by hyperuricosuria (11.2%), cystinuria (11.2%), cystinuria+hypocitraturia (11.2%) and cystinuria+hyperoxaluria (11.2%) in all metabolic screened children.

Stone analysis was performed on 8 children in group 1. Calcium (75%) and cystine stones (25%) were detected.

There was no significant difference between 1,25(OH)<sub>2</sub>D<sub>3</sub> serum levels and origin ( $p=0.094$ ), family history of stone disease ( $p=0.837$ ) in group 1 and 2. Serum Ca, P, Mg, ALP, PTH, urea, creatinine, HCO<sub>3</sub>, urine calcium/creatinine ratio, 24-hour urine calcium and 24-hour urine volume measurements were not also significantly correlated with 1,25(OH)<sub>2</sub>D<sub>3</sub>, respectively ( $r=-0.167$ ,  $r=-0.084$ ,  $r=0.224$ ,  $r=0.211$ ,  $r=0.223$ ,  $r=0.018$ ,  $r=0.011$ ,  $r=0.076$ ,  $r=0.163$ ,  $r=0.286$ ,  $r=0.114$ ).

## DISCUSSIONS

Urolithiasis is a multifactorial disease in which both genetic and environmental factors have effects on onset and severity. Urinary calcium excretion is an important factor in stone formation.<sup>1</sup>

In children, as in adults, calcium stones (calcium oxalate, calcium phosphate) are commonly found.<sup>4,7</sup> Ca stones are formed due to metabolic disorders and the most important reason is known as hypercalciuria.<sup>8-9</sup> In our study, we found Ca stone in 75% of the patients of whom stone analysis were performed. The stones

detected in our study were 92.8% renal, and 7.8% bladder+ureteral stone. Renal stones have been seen more frequently in Turkey recently.<sup>10</sup> This might be associated with the increase in development of our country.

When the cases with stone and hypercalciuria were evaluated according to their origin, it was found to be 52% West and 48% East origin ( $p=0.749$ ). The reason of no significant difference between patients of different regions might be because of the increase in migration from east to west in recent years and changes in dietary habits.

Many studies related to renal hypercalciuria found different results due to seasonal changes.<sup>11</sup> To eliminate the seasonal effects in our study all blood samples for examining 1,25(OH)<sub>2</sub>D<sub>3</sub> level were taken during the winter period.

Vitamin D, activated by hypophosphatemia and PTH, increases the risk of stone formation by increasing the intestinal Ca absorption and bone calcium mobilization and decreasing renal excretion which all cause increase in serum levels of Ca.<sup>12</sup> The effect of the VDR gene on stone formation could not be clarified yet. Güneş et al. observed a significant association between VDR gene polymorphism and family history and they concluded that the results were related to ethnic origin, environmental factors and technical differences in laboratory studies.<sup>13</sup> Even though there was no ultimate result about relationship between stone formation and polymorphism, genetic factor seem to be the major cause.<sup>6,14</sup>

In large epidemiological studies, familial transmission rate of 40-60% on idiopathic hypercalciuria (IH) has been identified.<sup>15-16</sup> Similarly, the history of urolithiasis and hypercalciuria in the families of patients with urolithiasis and hypercalciuria were 65% positive in our study. However, when the cases were examined in more detail, the history of family stone in group with urolithiasis were statistically more frequent compared to the group with hypercalciuria.

High urine Ca concentration and low urine volume cause stone formation with urinary supersaturation increased in distal nephron. In our study, it was detected that 24-hour urine Ca excretion was significantly higher in group with hypercalciuria than the group with nephrolithiasis ( $p=0.000$ ). The reason of the stone formation in the patients with low 24-hour urine calcium was based on low urine volume, low citrate and high oxalate-cystine excretion.

Kaplan et al. reported that plasma 1,25(OH)<sub>2</sub>D<sub>3</sub> level →

was not correlated with urinary Ca, P, phosphorus clearance, urine Ca and serum immunoreactive PTH.<sup>17</sup> In another study, serum Ca levels in patients with idiopathic hypercalciuria (IH) were normal, and PTH levels were normal or slightly low. In our study, there was no significant correlation between 1,25(OH)<sub>2</sub>D<sub>3</sub> levels and serum Ca, P, PTH, urinary Ca excretion in group with urolithiasis and hypercalciuria. Serum Ca and PTH levels were found normal in the study groups.

The pathogenesis of IH is not yet fully understood. The increase in intestinal Ca reabsorption or disturbance in Ca transport in enterocytes independent from D vitamin or increase in 1,25(OH)<sub>2</sub>D<sub>3</sub> production from kidneys as a response to disturbance in tubular Ca reabsorption were thought to play a role in the pathogenesis of hypercalciuria. Even if the real reason of intestinal Ca hyperabsorption is still not clear, vitamin D is thought to be the most important etiological factor.<sup>18-20</sup> In Kaplan's study when parathyroid function in patients with absorptive hypercalciuria (AH) was normal or suppressed, there was increase in 1,25(OH)<sub>2</sub>D<sub>3</sub> levels. Increased 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in patients with hyperparathyroidism was attributed to PTH which was effective in renal mitochondria

rather than circulating PTH, Ca and P concentrations. Maierfor et al. also found high levels of 1,25(OH)<sub>2</sub>D<sub>3</sub> in patients with IH.<sup>21</sup> Krieger et al. found normal 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in rats with genetically hypercalciuric and explained the increase in VDR levels bone-kidney-small intestine with the sensitivity to 1,25(OH)<sub>2</sub>D<sub>3</sub>.<sup>22</sup> We observed significantly higher 1,25(OH)<sub>2</sub>D<sub>3</sub> levels in groups with urolithiasis and hypercalciuria than the control group. However we did not detect a significant difference in 1,25(OH)<sub>2</sub>D<sub>3</sub> levels between the groups with or without stone. As we could not investigate the 25OHD<sub>3</sub>, we can not comment on the levels of body vitamin D or 1-alpha hydroxylase.

**Conclusions:** Increase in active vitamin D might have a role in the pathogenesis of urolithiasis and hypercalciuria. As no relationship was found between vitamin D levels and the other factors which were studied, it was thought that the genetic factors might be important in the increase in vitamin D.

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