# Reference values for urinary calcium, sodium and potassium in healthy newborns, infants and children

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The urinary calcium/creatinine ratio (UCa/Cr) in spot urine samples has been used extensively for screening and diagnosis of hypercalciuria (HC). The aim of this study was to determine the normal values for UCa/Cr, urinary sodium/creatinine (UNa/Cr), urinary potassium/creatinine (UK/Cr) and urinary sodium/potassium (UNa/K) ratios in healthy Turkish children aged 0-5 years. A total of 425 children were enrolled in the study. The urine samples were obtained from the second morning urine in children after breakfast and the first urine after feeding in infants. Urine Ca, Cr, Na and K levels were studied. A positive correlation was found between the UCa/Cr, UNa/Cr, UK/Cr and UNa/K ratios. Our results suggest that UCa/Cr is age-related and declines in the first five years of life except for in the newborn period. It might be concluded that determination of the upper limit of UCa/Cr in children less than five years old for every population can prevent unnecessary laboratory investigations and misdiagnosis of hypercalciuria.

Key words: calcium, creatinine, sodium, potassium, reference values, children.

Childhood idiopathic hypercalciuria (IH) is defined as urinary calcium excretion >4 mg/ kg/24 hours while on a regular diet<sup>1</sup>. Because of the difficulties in obtaining a 24-hour urine sample, the urinary calcium/urinary creatinine (UCa/Cr) ratio in spot urine samples has been used extensively for the screening and diagnosis of hypercalciuria (HC) (UCa/Cr >0.21 mg/mg)<sup>2,3</sup>. Previous studies showed that the UCa/Cr is age-related and declines in the first several years of life<sup>4-6</sup>. Furthermore, it was shown that high sodium (Na) and/or low potassium (K) excretion, which caused a high random urinary Na (UNa) to potassium (UK) ratio (UNa/K), is a risk factor in developing urinary stone disease in adults<sup>7</sup>. After that study, Osorio et al.8 demonstrated that UNa/K ratio had the strongest association with the UCa/ Cr ratio, suggesting the role of UNa and UK on the UCa/Cr ratio in children. Considering UCa/Cr values greater than 0.21 as HC, the prevalence of IH was reported to be between 4.2-5.8% in schoolchildren in different parts

of Turkey<sup>9,10</sup>. Since childhood urolithiasis is a common problem in our country, it is important to determine the reference values of UCa/Cr for children at different ages for early diagnosis and appropriate treatment. In clinical practice, we generally prefer to use UCa/Cr for monitoring Ca excretion in infants and young children, because of the difficulty in obtaining a 24-hour urine specimen from infants, but we were unable to find reliable reference values on the UCa/Cr ratio in Turkish infants and young children.

The aim of this study was to determine the agespecific reference values for UCa/Cr in healthy Turkish children aged 0-5 years, and to evaluate the relationship between UCa/Cr, urinary sodium/ creatinine (UNa/Cr), urinary potassium/creatinine (UK/Cr), and UNa/K ratios in the same group.

#### Material and Methods

A total of 425 healthy children aged 0-5 years including newborns born at Gazi University in the Obstetrics and Gynecology Department

and children aged 15 days-5 years followed up in Gazi University well-child clinics between August 2001 and February 2002 were enrolled into the study. Children with known kidney disorders, failure to thrive, any orthopedic problems or other causes of secondary HC or who were undergoing treatment with medications that affect UCa, as well as premature and low-birth-weight infants were excluded from the study. All children enrolled in this study were within normal limits for weight and height for their age and gender. All the newborn babies were breast-fed. No special dietary supplementation was given and no restriction was applied for other children. All the children were divided into six groups according to their ages: Group 1: term newborns less than 1 month, Group 2: infants between 1 month and <4 months, Group 3: between 4 and <9 months, Group 4: between 9 and <18 months, Group 5: between 18 months and <3 years, and Group 6: between 3 and <5 years. Since UCa/Cr variations are more prominent in infants, more groups under two years of age were designated. Urine samples were obtained from the second morning urine after breakfast in toilet-trained children during their visits to the well-child clinics. For children who were not toilet trained, urine samples were obtained by collection bags. In infants, spontaneously voided urine samples were collected after the first daily feeding. If any child, continent or incontinent, was unable to give urine samples during the hospital visit, parents were requested to collect or obtain the urine at home and to bring it to the hospital soon after voiding. At least two urine samples were taken from each patient in one week. Three ml urine was frozen to measure UCa and UCr, and these samples were studied by the same technician at the end of the study in the Research Laboratory of the Pediatric Nephrology Department. Urine Na and K were studied from the remaining fresh voided urine by the same technician in the Biochemistry Department. Because of the difficulty in obtaining adequate urine volume, UNa and UK could not be studied in all samples. Ratios of UNa/Cr (mEq/mg), UK/ Cr (mEq/mg), UCa/Cr (mg/mg) and UNa/K (mEq/mEq) were calculated for each subject. The 95th percentile for UCa/Cr was estimated as the upper limit of normal.

# Sample Analysis

Urine Na and K concentrations were determined by the iron-selective electrode method on the auto analyzer (Instrumentation Lab, Model 943) in the Biochemistry Laboratory. Urine Ca and Cr were measured manually by the chlorinic acid method on the spectrophotometer in the Research Laboratory of the Pediatric Nephrology Department.

Verbal inform consents to collect urine samples were obtained from parents. The ethics committee of the Gazi University School of Medicine approved the study.

# Statistical Analysis

Statistical analyses were performed using statistical software SPSS 9.0 programs. For each age group, 10th, 25th, 50th, 75th, 90th and 95th percentiles of UCa excretion were calculated. Data are presented as mean±standard deviation. The differences between groups were assessed by analysis of variance and paired t tests where appropriate. Otherwise, Kruskal-Wallis and Mann-Whitney U tests were performed when the results of analysis of variance showed that there were differences between the groups for UCa/Cr, and then multiple comparisons were done by Bonferroni. A p value < 0.05 was regarded as significant. The correlations between parameters were determined by Pearson correlation test.

#### **Results**

The study group included a total of 425 healthy children consisting of 168 girls (39.5%) and 257 boys (60.5%). The patients were divided into six groups: Group 1: 81 (19.1%) term newborns between 0 and 1 month; Group 2: 68 (16%) infants between 1 and <4 months; Group 3: 74 (17.4%) infants between 4 and <9 months; Group 4: 68 (16%) infants between 9 and <18 months; Group 5: 47 (11.1%) children between 18 months and <3 years; and Group 6: 87 (20.5%) children between 3 and <5 years. All the newborn babies were breast-fed. Thirty-nine of 88 infants between 1-6 months were exclusively breast-fed, 9 infants were fed only with formula and 40 were fed with both breast-milk and formula.

Comparison of the first and second urine samples showed no statistically significant difference (p>0.05). Table I shows the median

Table I. Median Values and the 10th, 25th, 50th, 75th, 90th and 95th Percentiles of the First and Second Urine UCa/Cr (mg/mg) in the Six Groups

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									Percentiles	ntiles					
		Give to	Cocond urine	10		25		20		75		06		92	Ì
Groups	n (%)	UCa/Cr Median + SD	DCa/Cr Median + SD	1st urine	2 <sup>nd</sup> urine	1st urine	2 <sup>nd</sup> urine	1st urine	2 <sup>nd</sup> urine	1st urine	2 <sup>nd</sup> urine	1st urine	2 <sup>nd</sup> urine	1st urine	2 <sup>nd</sup> urine
Group 1 0–1 month	81 (19.1)	81 (19.1) 0.075±0.16	$0.10\pm0.15$	0.02	0.03	0.04	0.05	0.07	0.10	0.19	0.19	0.31	0.41	0.56	0.52
Group 2 1-<4 months	68 (16)	0.35±0.32	$0.31\pm0.33$	0.07	0.08	0.16	0.18	0.35	0.31	0.54	0.53	0.84	0.95	1.18	1.05
Group 3 4-<9 months	74 (17.4)	0.25±0.25	$0.27\pm 0.25$	90.0	0.08	0.11	0.15	0.25	0.17	0.39	0.38	99.0	0.68	0.82	0.79
Group 4 9-<18 months	68 (16)	0.21±0.16	$0.19\pm 0.21$	0.07	0.03	0.11	0.08	0.21	0.19	0.29	0.28	0.49	0.55	0.64	0.67
Group 5 18 months-<3 years	47 (11.1)	0.17±0.15	$0.14\pm0.16$	90.0	0.04	0.09	0.08	0.17	0.14	0.25	0.28	0.37	0.43	0.59	0.59
Group 6 3-<5 years	87 (20.5)	0.10±0.079	0.11±0.071	0.03	0.03	0.05	90.0	0.10	0.11	0.14	0.15	0.23	0.23	0.28	0.25
(p>0.05).															

and mean values of the first and second urine UCa/Cr (mg/mg), and the 10th, 25th, 50th, 75th, 90th and 95th percentile of the first and second urine UCa/Cr (mg/mg) in the six groups. UCa/Cr ratio was significantly different among the groups (p<0.0001), and this difference was prominent in Groups 2 and 3, the values of which were significantly higher than in all the other groups. An inverse relationship was observed between age and UCa/Cr except in the newborn period in the 425 children (r = -0.205, p=0.0001). The UCa/Cr ratios in the newborn period were lower than those in other age groups. When the results of the newborns were excluded from the statistical study, an inverse and strong correlation was observed between UCa/Cr and age (r = -0.405, p = 0.0001).

Table II shows the median and mean of first and second UNa/K (mEq/mEq), UNa/Cr (mEq/mg) and UK/Cr (mEq/mg) ratios in all age groups.

When we evaluated the influence of nutrition on UCa excretion in the infants under six months, mean UCa/Cr was  $0.28\pm0.18$  in 39 breast-fed infants,  $0.51\pm0.33$  in 40 both formula and breast-fed infants and  $0.59\pm0.35$  in the formula-fed group. The mean UCa/Cr values in the breast-fed group were lower than those in the other two groups (p<0.05).

In our study, if the 95<sup>th</sup> percentile for UCa/Cr ratios in all age groups was considered as HC, the prevalence of IH was determined as: Group 1: 4 (5%); Group 2: 3 (4.4%); Group 3: 3 (4.1%); Group 4: 3 (4.5%); Group 5: 2 (4.2%); Group 6: 5 (5.7%); and overall 20 (4.7%). If we considered UCa/Cr values greater than 0.21 in all age groups as HC, the prevalence of IH was expected as 174 (40.5%).

Mean UCa/Cr was  $0.23\pm0.21$  in girls and  $0.24\pm0.21$  in boys, and there were no significant differences between the two genders (p>0.05).

A weak yet positive correlation was found between UCa/Cr and UNa/K (r=0.208, p<0.0001) and UCa/Cr and UNa/Cr (r=0.468, p<0.0001) (Figs. 1, 2). A weak and positive correlation was observed between UK/Cr and UCa/Cr (r=0.370, p=0.001) (Fig. 3).

# Discussion

Measurement of UCa has great importance in childhood since UCa excretion correlates directly with the prevalence of renal stone

Groups Age ( All Second Urine UNa/K (mEq/mEq), UNa/Cr (mEq/mg) and UK/Cr (mEq/mg) Ratios in and Table II. Median and Mean Values of First

				U	$U_{Na}/U_{\rm K}$							$U_{Na}$	$U_{Na}/U_{Cr}$							$U_{\rm K}\!/U_{\rm Cr}$	$U_{Cr}$			
		Firs	First urine			Secon	Second urine			First	First urine			Secon	Second urine			First	First urine			Secon	Second urine	
Groups	п	mean	n mean median SD	SD	u	mean	median	QS 1	u	mean	mean median	SD	u	mean	mean median	SD	u	mean	mean median	SD	u	mean	mean median	SD
Group 1: 0-1 month	89	2.01	68 2.01 1.43 1.76 65 1.74	1.76	65	1.74	1.58	1.60	65	2.12	0.85	3.16	89	2.60	1.02	4.20	69	1.35	0.82	1.44	69	1.40	0.85	1.34
Group 2: 1-<4 months	55	55 3.55	2.22	4.11	54	4.11 54 3.41	2.58	3.11	54	5.41	3.50	5.53	55	6.24	4.21	99.9	57	2.63	2.03	2.09	57	2.50	2.20	1.91
Group 3: 4-<9 months	67	67 2.45	1.66	1.66 2.72	29	2.08	1.56	2.39	29	3.86	2.00	3.94	29	5.42	2.26	10.1	69	2.88	2.11	2.42	69	2.73	2.28	2.35
Group 4: 9-<18 months	67	67 3.17	2.15	2.15 2.58 66	99	3.03	2.22	2.13	99	3.35	2.33	3.58	29	3.48	2.69	3.02	29	2.11	1.17	2.92	29	201	1.24	3.01
Group 5: 18 months-<3 years 46 2.58	46	2.58	2.22	2.06	47	2.22 2.06 47 2.23	1.92	2.03	47	2.45	1.81	2.93	46	2.92	2.39	2.98	47	1.44	1.09	1.06	47	1.34	1.12	1.10
Group 6: 3-<5 years	78	2.32	78 2.32 2.06 1.91 82 2.15	1.91	82	2.15	1.34	2.26	82	82 1.87	1.18	2.52	78	2.31	1.36	4.36	87	1.20	0.70	1.97	87	1.12	0.81	0.83
UNa/K: Urinary sodium/potassium. UNa/Cr: Urinary sodium/creatinine. UK/Cr: Urinary potassium/creatinine.	diun	1/potas	sium. U	Na/Ci	r: Uri	nary sc	odium/c	reatini	ne. L	JK/Cr:	Urinary	potas	sium	/creatir	nine.									Ì

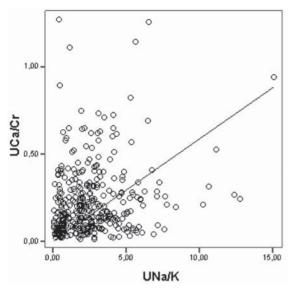


Fig. 1. Correlation between urinary calcium/creatinine (UCa/Cr) and urinary sodium/potassium (UNa/UK) in all age groups (r=0.208, p<0.0001).

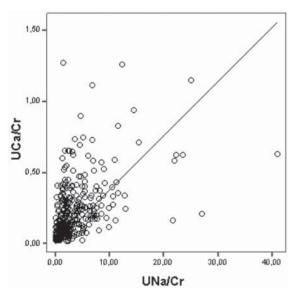


Fig. 2. Correlation between urinary calcium/creatinine (UCa/Cr) and urinary sodium/creatinine (UNa/Cr) in all age groups (r=0.468, p<0.0001).

disease. The urinary excretion of calcium in children has traditionally been evaluated by assessing the quantity of calcium in a 24-hour urine collection<sup>11-13</sup>. Assessment of UCa excretion by comparison of the urinary concentration of calcium to creatinine (UCa/Cr) in spot urine samples is well suited for children in whom 24-hour urine collections are difficult to obtain, especially in incontinent children. Moore et al.<sup>14</sup> first proposed the use of random

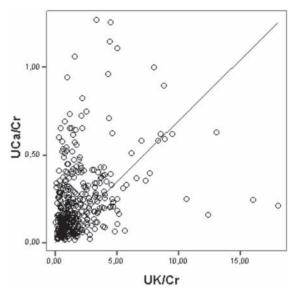


Fig. 3. Correlation between urinary calcium/creatinine (UCa/Cr) and urinary potassium/creatinine (UK/Cr) in all age groups (r=0.370, p=0.001).

UCa/Cr concentration ratio to screen for HC in children. Ghazali and Barratt<sup>12</sup> first showed that the second-morning urine samples after breakfast (post prandial UCa/Cr) were the most representative of 24-hour calcium excretion. Some authors recommended that an average of at least two urine samples should be accepted as the UCa/Cr level because of the variations in the random UCa/Cr levels, whereas others showed that only one second-morning urine sample was enough and sufficiently reliable to determine the UCa/Cr level because of the difficulties and high cost of obtaining more than one urine sample from children<sup>2,5,15,16</sup>. In the present study, two second-morning urine samples from the same child in one week were obtained and these two values were compared, and no statistically significant differences were found (p<0.05). Our results showed that only one second-morning urine sample was sufficient for reliability and was representative to determine the random UCa/Cr values.

According to the literature findings, UCa/Cr is higher in infants than in older children and adults<sup>2,4,5,15-18</sup>. The upper limit of normal for UCa/Cr in children under six years of age was twice that of adults<sup>2</sup>. In our study, the 95<sup>th</sup> percentile for UCa/Cr in infants and children under five years of age was very close to the commonly accepted upper limit of normal for representative groups from published reports

(Table III)<sup>2,4-6,19</sup>. It was also demonstrated that there was an inverse relationship between UCa/Cr and age except in the newborn period; UCa/Cr values approached 0.21 in the group aged 3 to <5 years, which is accepted as the upper limit for adults. Some authors have demonstrated similar results<sup>5,6,17,18</sup>. The small differences between the results of these studies could be due to the differences in the methods and the timing of urine sampling. Increased UCa/Cr in infants and young children may be due to the decrease in excretion of creatinine per unit of lean body mass in this age group<sup>2</sup>. Furthermore, some authors suggested the effect of consuming large amounts of milk and dairy products on higher UCa/UCr levels in infancy<sup>6,16</sup>. In the present study, the UCa/Cr ratios in the newborn period were lower than those in other age groups. When the values of the newborn period were included, an inverse and weak correlation was observed between UCa/Cr and age in the 425 children (r = -0.205, p=0.0001). However, when the newborns were excluded from the study, an inverse and strong correlation was observed between UCa/Cr and age (r= -0.405, p=0.0001). Reusz et al. 16 and Siegel et al.<sup>19</sup> showed similar results in the newborn period.

The majority of the studies on UCa/Cr excretion showed no differences between genders<sup>5,6,20</sup>, whereas some authors have reported higher UCa/Cr values in boys than girls<sup>21</sup>. No significant differences were observed between girls and boys in the present study (p>0.05). The importance of sodium intake as a factor that influenced UCa excretion and the rate of HC were first demonstrated by Aladjem et al.<sup>22</sup>. Osorio et al.<sup>8</sup> then determined a positive correlation between UCa/Cr and UNa/K, and the value of UNa/K was greater than 4.5 in HC children. In addition, Cirillo et al.<sup>7</sup> showed a positive correlation between UNa/K, UNa/Cr and urinary stone disease in adults. However, some other studies did not support those findings<sup>5,23</sup>. Similar to what has previously been reported in hypercalciuric children, a weak yet positive correlation was found between UCa/Cr and UNa/K as well as between UCa/Cr and UNa/Cr in our study.

Vachvanichsanong et al.'s<sup>4</sup> results were similar to ours. These results suggested that increased salt intake promotes UNa and UCa excretion. Finally, all of these effects suggest the importance of low

Table III. UCa/Cr (mg/mg) Ratios Reported in Different Studies

	Present study	Sargent et al. <sup>2</sup>	Vachvanichsanong et al. <sup>4</sup> Matos et al. <sup>6</sup> So et al. <sup>5</sup>	Matos et al. <sup>6</sup>	So et al. <sup>5</sup>
Year	2002	1993	2000	1997	2001
Population	Turkish	American	Thai	Switzerland.	African-American, Caucasian
Number of subjects	425	215	488	410	368
Defined upper limit of normal	95 <sup>th</sup> percentile	95th percentile	95 <sup>th</sup> percentile	95th percentile 95th percentile	95 <sup>th</sup> percentile
95th percentile of UCa/Cr in different age groups	0-30 days; 0.52 1-4 mos; 1.05 4-9 mos; 0.79 9-<18 mos; 0.67 18 mos-<3 yrs; <0.59 3-<5 yrs; <0.25	<7 mos; 0.86 19 mos-3 yrs; 0.42 Adult; 0.22	<pre>&lt;6 mos; 0.75 1-&lt;2 yrs; 0.40 5&lt;10 yrs; 0.29</pre>	<12 mos; 0.81 <7 mos; 0.86 1-2 yrs; 0.56 8-18 mos; 0.6 2-3 yrs; 0.5 19 mos-6 yrs; 3-5 yrs; 0.41 7-16 yrs; 0.22	<7 mos; 0.86 8-18 mos; 0.6 19 mos-6 yrs; 0.42 7-16 yrs; 0.22

sodium intake for preventing the occurrence of HC and renal stone disease. On the other hand, the inverse relationship between UK and UCa excretion and the positive effect of potassium intake on the calcium balance were reported in some articles<sup>7,8</sup>. An increase in dietary potassium reduces UCa excretion, and it is suggested to have a direct or indirect effect in the promotion of renal calcium retention<sup>8</sup>. An increase in dietary potassium reduces UCa excretion and causes calcium balance to become more positive, suggesting that potassium either directly or indirectly promotes renal calcium retention and inhibits net bone resorption<sup>24,25</sup>. On the other hand, potassium can cause renal phosphate retention, which inhibits renal synthesis of calcitriol and, subsequently, intestinal calcium absorption<sup>26</sup>. Contrary to what has previously been reported in HC children, no significant relationship was found between UK/Cr and UCa/Cr in healthy children in our study. Thus, it appears that the child with idiopathic HC may differ from the normal child by exhibiting a higher sensitivity to the dietary intake of sodium and potassium8. Although geometric means of UNa/K of African-American and Caucasian children in different age groups were similar, Ca/Cr was higher in African-Americans, indicating the role of other factors in determining UCa/Cr<sup>5</sup>. UK/Cr ratios were lower in the newborn period than in those in the "one month to <2 years" group. Furthermore, serum potassium level is higher and urinary potassium excretion is lower in the newborn period. The lower level of UK/UCr in the first month of life might be due to the lower excretion of potassium and higher excretion of creatinine secondary to the tubular immaturity and the effect of maternal creatinine.

In our study, if the 95<sup>th</sup> percentile for UCa/Cr ratios in all age groups were considered as HC, the prevalence of IH was determined as 4.7% in healthy children. However, if we considered the UCa/Cr values greater than 0.21 in all age groups as HC, the prevalence of IH was expected as 40.5%. Considering the UCa/Cr values greater than 0.21 as HC, the prevalence of IH was reported to be between 4.2-5.8% in schoolchildren in different parts of Turkey<sup>9,10</sup>. The present study highlights the importance of determining the reference values of UCa/Cr for children of different ages for early and proper diagnosis and appropriate treatment of HC.

Since all the newborns were breast-fed, we could not evaluate the relationship between nutrition and the UCa/Cr levels in this age group. UCa/Cr excretion in infants aged 1-6 months was compared according to their feeding patterns. We found that UCa/Cr excretion in breast-fed infants was lower than in both formula- and breast-fed infants and only formula-fed infants. However, it could not be ascertained whether formulas influence the UCa/Cr because of the limited number of infants in the formula-fed group. On the other hand, Lambrecht et al.<sup>27</sup> investigated the role of calcium utilization of intestinal flora in urinary calcium excretion and found that calcium utilization of intestinal flora does not have a distinct effect on UCa excretion.

In conclusion, our results indicate an inverse relationship between UCa/Cr and age. Determination of the reference values for the UCa/Cr ratios in the same geographical area, ethnic background, race and age is very important for screening for IH and for taking preventive measures against urinary tract stone formation. If possible, diagnosis of HC should be confirmed with a 24-hour urine specimen in a child whose spot UCa/Cr exceeds the 95th percentile. Although the most important complication of hypercalciuria is renal stones, renal and urinary tract ultrasonography was not performed in any of the patients. Further large-scale studies including renal and urinary tract ultrasonography are warranted. Our results also suggest that obtaining only one spot urine sample was simple, reliable, cost-effective and representative for screening the random UCa/Cr values. Finally, our findings might be interpreted as indicating the importance of a low-sodium intake to prevent the occurrence of IH and urinary tract stones. The impact of the positive correlation between UNa/Cr and UCa/Cr and the effect of different feeding patterns on IH development and stone formation in childhood need to be investigated further.

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