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Determination of upper reference value of urinary calcium-creatinine ratio for the paediatric population in Burdwan district

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ABSTRACT

To estimate the rate of excretion of urinary calcium, a 24-hour sample of urine is required and this is not always easy to collect accurately in infant and children. So, random urine calcium to creatinine ratio (Ca/Cr ratio) has been developed. But as the ratio varies worldwide, reference values of the parameter in paediatric population are not developed. To determine reference value, the present study was conducted in healthy paediatric population in Burdwan district, West Bengal. This study was performed on 693 healthy paediatric subjects, aged between 3 months to 18 years and divided into five groups. Early morning non-fasting urine samples from all study groups were analyzed for Ca/Cr ratio. A negative correlation was observed between age and urinary Ca/Cr ratio, but there was no significant difference of urinary Ca/Cr ratio between two sexes. Considering 97.5th percentile of the underlying distribution of values as the upper limit of reference range, upper reference values of urinary Ca/Cr ratio for age groups of <1, 1 -< 2, 2 -< 5, 5 -< 10 and 10 - 18 years in mg/mg of 1.61, 1.38, 1.13, 0.89 and 0.71 respectively were derived. As the upper reference values of urinary Ca/Cr ratio of different age groups in present study are different from other previous studies conducted in different areas, we conclude that upper reference value of urinary Ca/Cr ratio should be estimated in every geographic area.

Keywords: Urinary Calcium-Creatinine Ratio; Hypercalcuria; Reference Range

1. INTRODUCTION

The prevalence of idiopathic hypercalciuria (IH) has been reported to vary between 2.9% - 6.2% in the age group of 3 months to 18 years [1-6]. The majority of these cases are asymptomatic. Symptomatic cases have various manifestations such as gross hematuria, dysuria, nocturnal enuresis, urinary frequency—urgency syndrome, supra-pubic and abdominal pain and recurrent urinary tract infections [7]. It predisposes to nephrolithiasis that may cause renal damage. These complications are avoidable if appropriate preventive measures are undertaken by early detection of IH. It is done by measurement of urinary calcium excretion, but it is difficult to take the accurate 24 hours urine in young and non-toilet trained children. Therefore, hypercalcuria can be screened by random urine Ca/Cr ratio [8-10] preferably fasting. It is incumbent on a laboratory to report urinary Ca/Cr ratio, and a reference interval must be required above which a result is considered abnormal, but these cut-offs are not firmly established because of difficulties of generating reference intervals for children particularly across the span of ages in childhood [11]. Reference values for urine calcium/ creatinine ratio in normal infant and children have been reported by several authors worldwide, but these data are controversial, possibly due to the differences between study populations, genetic characteristics and the variations in dietary intake of calcium, sodium and protein in different geographic regions [1-4,9, 10,12-15]. So the objective of the present study was to determine urine Ca/Cr ratio in healthy infant and children in Burdwan district, West Bengal.

2. MATERIAL AND METHODS

2.1. Selection of Subjects

A total of 693 normal infant and children aged 3 months to 18 years from three public schools and a well-baby

clinic of Burdwan district were selected by simple random sampling after informed consent had been received from the parents between March 2011 and May 2013. Among them three hundred twenty (53.82%) were boys and 195 (46.18%) were girls All of them had normal physical growth and blood pressure. They were told not to change their eating habits and physical activities. Children with conditions that would influence urinary calcium excretion such as being on medical treatment or suffering from malnutrition, chronic disease such as kidney diseases, metabolic disorders were excluded. Then the study population was divided into five groups according to their age: Group I: <1 year, Group II: 1 -< 2 years, Group III: 2 -< 5 years, Group IV: 5 -< 10 years and Group V: 10 - 18 years.

2.2. Collection of Samples

Early morning non-fasting urine sample were collected from the study population to determine calcium and creatinine levels. After collection all the tests were done immediately.

2.3. Parameters Assay

Urinary calcium was estimated by 2-cresolphthalein complexone method [16] using semi auto analyser (Chem $5v_2$ plus). Intra assay CV% was 1.5 and inter assay CV was 2.7 for this method. Urinary creatinine concentration was assayed by modified Jaffes' method [17] using (Chem $5v_2$ plus) semiautoanalyser. Intra-assay CV% was 1.8 and inter-assay CV% was 2.9. Then urinary Ca/Cr (mg/mg) ratio was calculated.

2.4. Statistical Analysis

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows.

3. RESULT

In Burdwan district the distribution of urinary Ca/Cr ratio in the paediatric study population is depicted in **Figure 1.**

Table 1 showed the mean, standard deviation and percentile values of different age group. It was found that there was decrease in urinary Ca/Cr ratio as age of the study population increased and this ratio significantly negative correlate (Pearson Correlation −0.217) with increasing age ($p < 0.01$) analysed by Pearson correlation. It was also seen that different percentile values of each age group also decreased according to increased age.

Although urinary Ca/Cr ratio and its 97.5th percentile value were more in girls, the different did not show significant difference ($p > 0.01$) as shown in the **Table 2.**

Figure 2 shows the comparison between the percentile values of different age groups and it is shown that there was definite decrease in percentile values according to increasing of age.

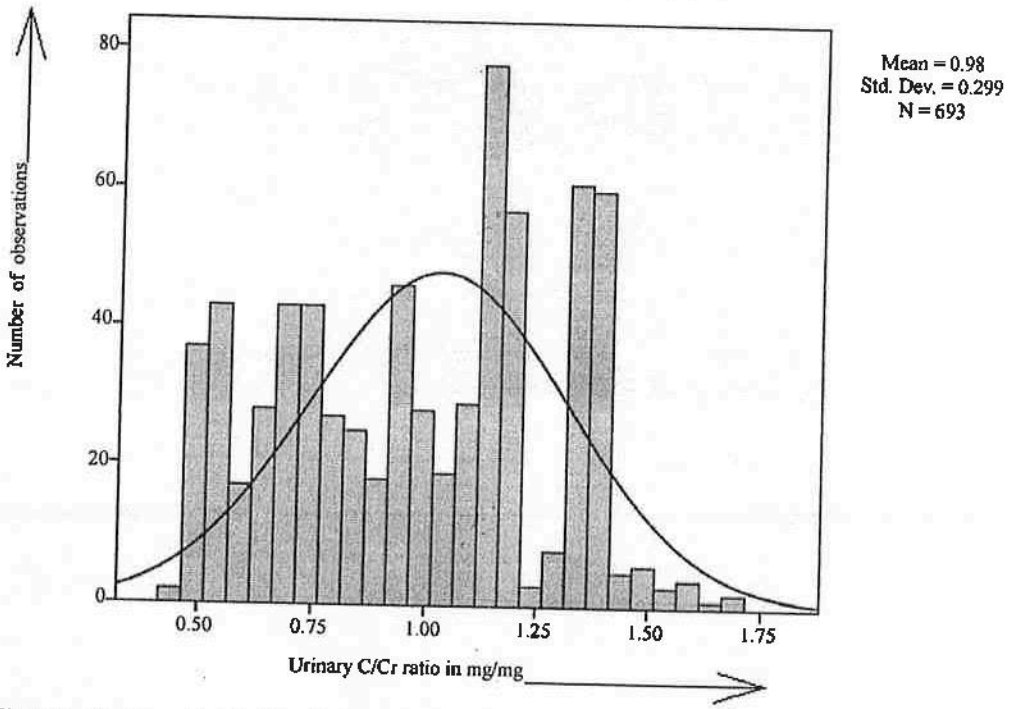

Figure 1. Frequency distribution of urinary Ca/Cr ratio in paediatric study population in Burdwan district.

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Table 1. Ca/Cr ratio in urine and its percentile values of different age group.

Age group (year)	n	Urinary Ca/Cr ratio (mg/mg)	25th percentile	50th percentile	75th percentile	95th percentile	97th percentile
<1	139	1.38 ± .073	1.35	1.37	1.39	1.58	1.61
1 -< 2	155	1.17 ± 0.06	1.15	1.16	1.18	1.36	1.38
2 -< 5	121	0.99 ± 0.07	0.93	0.97	1.04	1.10	1.13
5 -< 10	133	0.77 ± 0.07	0.72	0.76	0.82	0.88	0.89
10 - 18	145	0.57 ± 0.07	0.51	0.54	0.64	0.69	0.71

Value are mean ± SD; n = number of cases in each group.

Table 2. Urinary Ca/Cr ratios of study population based on different age groups and sex.

Age group (year)	Sex	n	Urinary Ca/Cr ratio (mg/mg)	97.5th percentile	Pearson correlation between both sex within each age group
<1	Boys	76	1.37 ± 0.05	1.54	
	Girls	63	1.40 ± 0.09	1.68	p > 0.05
1 -< 2	Boys	82	1.16 ± 0.06	1.38	
	Girls	73	1.18 ± 0.06	1.38	p > 0.05
2 -< 5	Boys	67	0.98 ± 0.06	1.12	
	Girls	54	1.0 ± 0.07	1.17	p > 0.05
5 -< 10	Boys	65	0.74 ± 0.06	1.02	
	Girls	68	0.82 ± 0.07	1.07	p > 0.05
10 - 18	Boys	83	0.52 ± 0.03	0.63	
	Girls	62	0.63 ± 0.05	0.65	p > 0.05

Values are mean ± SD; n = number of cases in each group; p > 0.05 consider statistically insignificant.

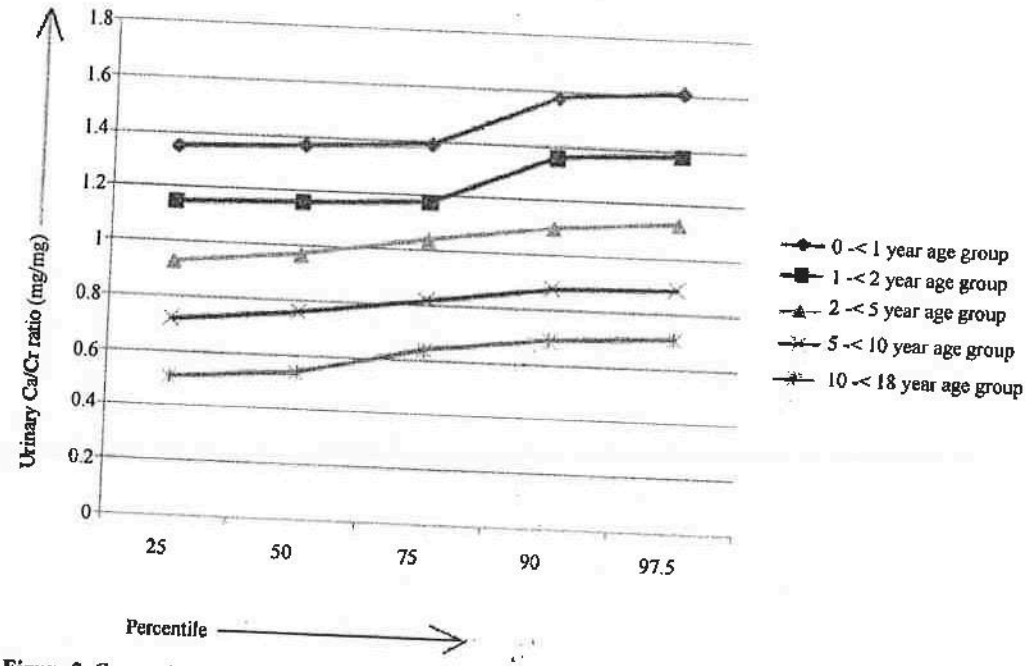

Figure 2. Comparison of percentile values of different age groups in present study population.

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After calculating the upper reference of urinary Ca/Cr ratio, it was found that upper reference value of this parameter has got inverse relationship with age as observe in the **Table 3**.

4. DISCUSSION

In children urinary Ca/Cr ratio is a useful and reliable method for determining hypercalcuria and also is a non-invasive and relatively inexpensive method [18]. But reference interval of this parameter for the paediatric age group is not yet established as it is dependent on several factors such as age, geographical area. So the present study was conducted to establish a reference interval in our study area and it was first found that there was a significant inverse relationship between urinary Ca/Cr ratio and age. This finding was well corroborated with several previous studies [9,16,19-22]. It also observed that for every age group, different urine Ca/Cr ratio was reported [3,8,23]. This difference may be due to exposure to sunlight, climate, genetics, nutritional habits, drinking water and ethnicity. In respect to relationship between Ca/Cr ratio of urine and age, another important observation was that same as few older studies [8], there was higher level of urinary Ca/Cr ratio in infant than other age group. It is may be secondary to low creatinine excretion per unit body mass [8] and also tubular immaturity to excrete creatinine [11]. If sex factor is considered, as previous reports it was found that there was no significant difference in urinary Ca/Cr ratio between both sex [9,13,19,22]. As for most analytes, the lower and upper reference limits are assumed to demarcate the estimated 2.5^{th} and 97.5^{th} percentiles respectively, of the underlying distribution of values [24], percentile values of urinary Ca/Cr ratio were calculated. 95^{th} and 97.5^{th} percentile for urinary Ca/Cr ratio of different age groups in present study in this geographic area was the same as some reports and different with other [1,6,9,10,14-15,18-23,25-29]. Then upper reference value of urinary Ca/Cr ratio of different age group were found and this to some extent different from one previous study [11]. Despite the disagreement between the upper reference value in this report and other, the accumulated data support the higher and changing upper reference value that have been adopted for use in day-to-day clinical practice at this institution.

Table 3. Determined upper reference value of urinary Ca/Cr ratio in different age groups.

Age (year)	Upper reference value (mg/mg) of urinary Ca/Cr ratio
0 -< 1	1.61
1 -< 2	1.38
2 -< 5	1.13
5 -< 10	0.89
10 - 18	0.71

5. CONCLUSION

The present study showed parallel to other studies that urinary Ca/Cr ratio values of different age groups may differ according to geographic location. So, for screening of hypercalciuria, upper reference values of random urinary Ca/Cr ratio in different paediatric age groups should be determined in each geographic location.

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