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Determination of reference interval (RI) of spot urinary oxalate to creatinine ratio in children of pakistani origin under six years of age: A cross-sectional study☆

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ABSTRACT

Background: The gold standard screening method of hyperoxaluria in children is using 24-hour urine collection. Urine collection may be cumbersome and challenging for children. Reference intervals (RI) of oxalate for the Pakistani population are not readily available. Therefore we aimed to determine the oxalate to creatinine ratio (Ox: Cr) for Pakistani children <6 years of age.

Materials and Methods: A cross-sectional study was conducted at Aga Khan University from June 2018 to October 2019. Random urine samples from apparently healthy children < 6 years were collected and stored at -30°C until analysis after adding 6M HCl. Oxalate was measured on Micro lab 300 using a kit based on oxalate oxidase principle, while creatinine was measured by kinetic Jaffe reaction. Data was analyzed by EP evaluator and SPSS 23. Ox: Cr ratio was calculated and reported with 90% confidence interval (CI) and interquartile range (IQR).

Results: The mean age of study subjects (n=120) was 29 ±22.3 months with an M: F ratio of 1:1. Children of various ethnicities were included from all over Karachi. The majority of the subjects were Urdu speaking (37.5%). Median Ox: Cr was 0.13(0.10). No significant difference was noted in the median Ox: Cr ratio between various ethnicities (p>0.05). It was significantly different in group I to V which was 0.25 (IQR: 0.06), 0.19 (IQR: 0.11), 0.15 (IQR: 0.04), 0.11 (IQR: 0.06) and 0.08 (IQR: 0.04) respectively (pvalue <0.001).

Conclusion: The established RIs of Ox: Cr ratio was 0.05-0.34 (90% CI). Ox: Cr ratio showed a declining trend with age. Large scale reference interval studies are encouraged, taking diet and age into consideration.

1. Introduction

Oxalate is a highly insoluble end product of ascorbic acid and amino acids glycine, serine, and hydroxyproline in humans. It is mainly excreted via kidney in the form of calcium salt, with a tendency to crystallize in the renal tubules. Increase urinary oxalate excretion (Hyperoxaluria) can result from inherited disorders of oxalate metabolism, intestinal diseases or oxalate rich diet. The overproduction of oxalate in the liver results in increased excretion by the kidney which can lead to nephrocalcinosis, urolithiasis and even chronic kidney disease [1].

Reference intervals (RI) of oxalate for Pakistani population are not readily available [2] and the values used by most labs are usually taken from the literature from studies mainly performed on Caucasian or from manufacturer package insert of oxalate reagent kits. RI of an analyte assist the clinician in differentiating between health and disease [3]. It is established by testing healthy population and figuring out what appears to be "normal" for them after defining the reference population demographically. Careful determination and verification or validation of RI by the laboratory for use is important to ensure proper utility.

Children with recurrent stone formation need comprehensive metabolic evaluation for serum and urine chemistries and chemical

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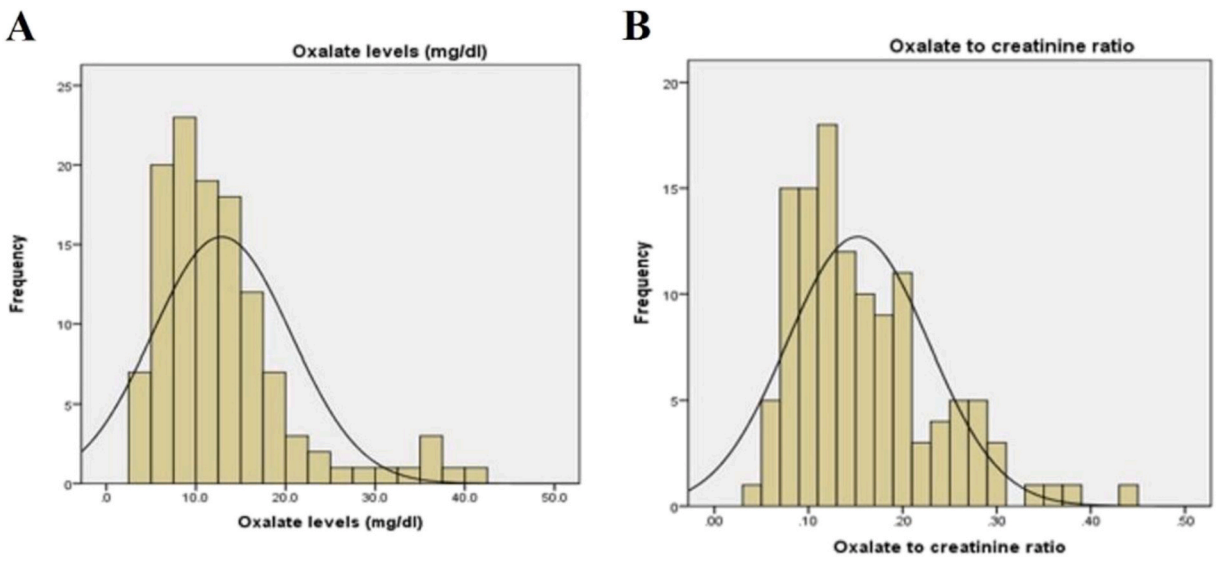

Fig. 1. Overall distribution of urinary oxalate levels (1a) and Ox:Cr ratio (1b) in apparently healthy children less than six years of age (n = 120).

composition of stone to identify the factors predisposing to stone formation [4,5]. In this regard, undifferentiated hyperoxaluria is seen in up to 43% of Pakistani pediatric stone formers [6]. The interpretation of biochemical information is critical for proper diagnosis and hence management protocol for the patient. Therefore, locally derived RI are needed for diagnosis and also for monitoring patient [7]. However, it is troublesome and inaccurate to collect 24-h urine volumes in infants and toddlers, as they do not void spontaneously. In children <6 years, urine collection is usually performed by bladder catheterization, which is an invasive procedure and increases the risk of urinary tract infection. Urinary oxalate measurement in children is important for assessing inherited disorders of oxalate metabolism as they usually manifest in early childhood and determining population specific RI of oxalate in spot urine specimen is expected to help in diagnosis and management of inherited disorders of oxalate metabolism. Therefore, we aim to determine the spot urine oxalate to creatinine reference interval in children under 6 years of age for our population.

2. Materials and methods

A cross sectional study was conducted at Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi from June 2018 to October 2019 after approval from the ethical review committee of Aga Khan University Hospital. Inclusion criteria included children less than 6 years of age, not suffering from any illness during last two weeks and no hospitalization since last four weeks. Children on vitamins or with either past history of kidney stones or family history of kidney stones were excluded. A standardized questionnaire was used and recommended target sample size was calculated using CLSI Guidelines C28-A3. Non-probability (consecutive sampling) was done.

Parents or guardian of each reference individual were invited to participate in the study. An explanation of the study, consent form (written in the local language, in accordance with the guidelines of the local ethics committee) and the procedure for participation was explained to the parents by primary investigator. Those who consented were provided with a container for urine collection and procedure for collection was explained. A 5 ml random morning urine sample was collected and stored at −30 °C until analysis after adding 6 M hydrochloric acid.

Urinary Oxalate was measured on Micro lab 300 using a kit based on oxalate oxidase principle by Trinity Biotech Plc, Wicklow, Ireland while creatinine was measured by kinetic Jaffe reaction on ADVIA 1800 by

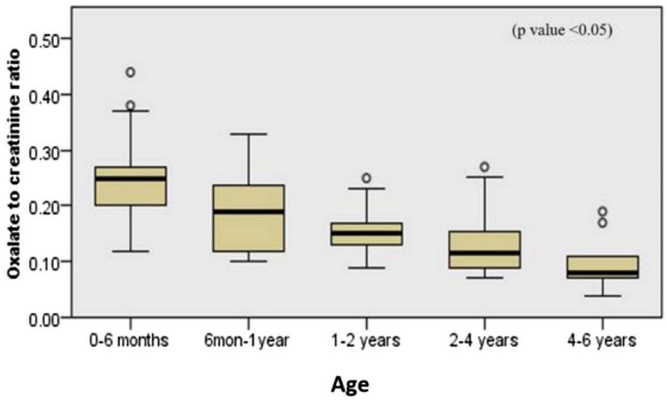

Fig. 2. Box and whisker plot depicting declining trend of Ox:Cr with advancing age of children (age range <6 years).

Siemens Diagnostic, USA. Samples were analyzed in batches with quality control specimen for validating the results. Oxalate to creatinine ratio (Ox:Cr) was calculated. Clinical Laboratory of Aga Khan University Hospital is accredited by College of American Pathologist, USA.

Data was analyzed by EP evaluator and SPSS 23. Subjects were categorized based on ages into five groups. Group 1 (0–6 month), Group 2 (6 mon-1 year), Group 3 (1–2 year), Group 4 (2–4 year) and Group 5 (4–6 year). Descriptive data was analyzed for frequencies and median with IQR. P value < 0.05 was taken as statistically significant.

Our study has been reported in line with the STROCSS criteria [8].

Our study was registered in ClinicalTrials.gov with registration ID: NCT04756024 publicly accessible via:

https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S000AOWW&selectaction=Edit&uid=U0005AWR&ts=2&cx=-hwngbp.

3. Results

One hundred and twenty apparently healthy subjects <6 years of age were included. Mean age of study subjects (n = 120) was 29 ± 22.3 months, with equal gender distribution (M: F 1:1). Subjects from various districts of Karachi were enrolled; majority were from District East (30.8%) followed by Central (28.3%), West (21.7%), Malir (15%) and (4.2%) from South. The study population constituted of diverse ethnic

Table 1
Comparison of urinary Ox: Cr cutoffs from published literature.

Country	Study type	n	Age	Urine sample	Method	Results
India [14]	Cross-sectional	208	8–15 years	24 h	Chromotropic acid reaction	0.7 mg/kg (median) 1.5 mg/kg (95th percentile) of 24-h oxalate. Ox/Cr 0.01(median) and 0.06 (95th percentile)
Poland [15]	Cross-sectional	109	6–16 years	Second morning urine sample	ND[a]	Ox/Cr (mmol/mmol) <12 years 0.076 (95th percentile) Ox/Cr (mmol/mmol) >12 years 0.051 (95th percentile)
Switzerland [16]	Cross-sectional	384	1 month-17 years	Second morning urine sample	Enzymatic using oxalate oxidase	95th percentile for Ox/Cr (mg/mg) 0–0.5 years < 0.175 0.5–1 years <0.139 1–2 years <0.103 2–3 years <0.08 3–5 years <0.064 5–7 years <0.056 7–17 years < 0.048
Turkey [17]	Cross-sectional	953	6–15 years	Non fasting random urine	Enzymatic using analytical procedure on manual spectrophotometer	Ox/Cr (mg/mg) (mean ± SD) 6–9 years 0.023 ± 0.015 0.048 (95th percentile) 10–12 years 0.018 ± 0.012 0.042 (95th percentile) 13–15 years 0.016 ± 0.011 0.042 (95th percentile)
Current Study	Cross-sectional	120	<6 years	Non fasting random urine	Enzymatic using oxalate oxidase	Ox/Cr (mg/mg) [median (IQR)] 0–6 months 0.25 (IQR: 0.06) 6months-1 years 0.19 (IQR: 0.11) 1–2 years 0.15 (IQR: 0.04) 2–4 years 0.11 (IQR: 0.06) 4–6 years 0.08 (IQR: 0.04)

[a] ND: Not determined.

background majority being Urdu speaking (n = 45, 37.5%) followed by Pakhtoon (n = 27, 22.5%), Punjabi (n = 24, 20%), Sindhi (n = 19, 15.8%) and Baloch (n = 5, 4.2%).

Data was not normally distributed for oxalate and Ox: Cr by Kolmogorov test (p value < 0.05) as shown in Fig. 1a and b and therefore median (IQR) was reported.

The overall median Ox:Cr ratio for children <6 years of age was 0.13 (0.10). At 90% CI, the RI for spot Ox:Cr ratio was 0.05–0.34. The Ox:Cr ratio was highest in group I with a median (IQR) of 0.25 (0.06). Declining trend of the ratio was noted for age from Group II-V with median Ox:Cr ratios of 0.19(0.11), 0.15(0.04), 0.11(0.06) and 0.08 (0.04) respectively. Significant difference was noted in median Ox:Cr in group I to V respectively (p-value <0.001), as shown in Fig. 2.

Differences were not significant in median (IQR) Ox:Cr between various ethnicities (p value > 0.05)

4. Discussion

Reference interval and cut-off constitute an essential component of a lab report. RI in the healthy state are affected by several factors including body size, age, ethnicity, gender, diet, environmental conditions and hence population specific RI are preferred as clinical decisions are based on them. In patients with stones, urinary excretion rate of some solutes like calcium and oxalate, is usually performed to investigate the underlying cause [9,10]. The precise quantification and interpretation of oxalate is dependent on multiple factors including body mass, dietary habits, environmental conditions and lifestyle, which for Pakistani are much different from the Caucasians [11] and hence the need to establish RI for the desired population [12].

In primary hereditary Hyperoxaluria (oxalosis), stone formation usually begins in childhood, with 65% of the cases occurring before the age of 12 years. There is a tendency of stones to recur at intervals and without treatment, more than 80% of the patients with oxalosis become uremic by 20–30 years of age. Treatment with pyridoxine reduce endogenous oxalate production as well as stone progression and may prevent them all together. Therefore, early diagnosis is essential for proper management. After a thorough search of medical literature, no local study was published that has established the RI of oxalate in our population while the diagnosis of oxalosis rests on the findings of increased urinary oxalate excretion. Studies conducted in Pakistan revolves around calcium oxalate stones and their prevalence [13]. There are few studies which have investigated oxalate excretion in pediatric population. We also reviewed published literature on urinary Ox:Cr in healthy children have using following search engines; Pubmed, Medline and Scholar Google (Table 1) [14–17]. Results from these studies are not consistent and variable Ox:Cr ratio are reported. This variations in urinary oxalate excretion in different studies, could be due to usage of different laboratory or urine collection methods or different dietary and life style factors. The urinary Ox: Cr ratio declines with age and shows variability according to geographic area [18]. This validates the need of country specific Ox:Cr ratio RI [19].

In this study, we determined values for the Ox:Cr ratio in healthy children less than 6 years of age. Results from current study are in close proximity to study done on children stratified in seven age groups with age range of 1 month–17 years [16]. An inverse statistically significant relationship between the Ox:Cr ratio and age was identified, which is similar to what scientists have reported in healthy Turkish school children (n = 953) [17]. The strength of this study is that it included participants from different ethnic groups residing in Pakistan. However, it cannot be applied universally. In the current study dietary habits of children, environmental conditions, body composition were not assessed. We propose that further studies should be conducted keeping in view of these important parameters [20–22]. Despite all these limitations, a good data set of a RI for morning urine Ox:Cr ratios was provided with this study. This could be useful for identifying patients with Hyperoxaluria.

5. Conclusion

Based on our study findings, Ox:Cr ratio showed a declining trend with age in children. In children <6 years of age RI for Ox:Cr was calculated as 0.05–0.34. Interpretation of random urine Ox:Cr ratios is challenging in children and large scale reference interval studies are encouraged taking diet and age into consideration.

Every country should have its own normal reference values to determine the underlying metabolic risk factor for kidney stone disease, since regional variation in the dietary intake of proteins and other nutrients can affect normal urinary excretion of oxalate.

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Ethical approval

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Author contribution

SBH, AHK & SQ: Conception, design of study and manuscript writing.

JT & AHK: Drafting the work and revising it critically for important intellectual content. Also final approval of version to be published.

LJ, HM & SBH: Data collection and literature review.

Registration of research studies

Name of the registry: ClinicalTrials.gov.

Unique Identifying number or registration ID: NCT04756024.

Hyperlink to your specific registration (must be publicly accessible and will be checked):https://register.clinicaltrials.gov/prs/app/action/SelectProtocol?sid=S000AOWW&selectaction=Edit&uid=U0005AWR&ts=2&cx=-hwngbp.

Guarantor

Dr.Aysha Habib Khan.

Consent

A copy of the written consent will be available for review by the Editor-in-Chief of this journal on request.

Declaration of competing interest

There is no conflict of interest.

Acknowledgement

Nil.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.amsu.2021.102251.

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