Initial collection of an inadequate 24-hour urine sample in children does not predict subsequent inadequate collections

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Summary Table: Child demographic and parental socioeconomic characteristics of incident stone formers with 24-hour urine collections (n=366)

Variable	Overall (N=366)	Adequate Collection (N=80*)	Inadequate Collection (N=286)	p-value
Race • African-American • Caucasian • Other • Missing/Refused/Unknown	 8 (2.1%) 357 (95.0%) 5 (1.3%) 6 (1.6%)	 1 (1.3%) 77 (96.3%) 2 (2.5%) 0 (0%)	 5 (1.7%) 273 (95.5%) 2 (0.7%) 6 (2.1%)	0.3
Sex • Female • Male	 206 (54.8%) 170 (45.2%)	 48 (60.0%) 32 (40.0%)	 151 (52.8%) 135 (47.2%)	0.3
Stone procedure • Yes • No	 139 (37.0%) 237 (63.0%)	 33 (41.3%) 47 (58.8%)	 103 (36.0%) 183 (64.0%)	0.4
Age (years)	11.1 ± 3.9	11.9 ± 4.2	10.9 ± 3.8	0.1
BMI Percentile	54.1 ± 35.0	51.8 ± 38.1	54.7 ± 34.2	0.5
Insurance Category • Private • Public • Self-Pay or Missing	 245 (65.2%) 111 (29.5%) 20 (5.3%)	 49 (61.3%) 25 (31.3%) 6 (7.5%)	 192 (67.1%) 80 (28.0%) 14 (4.9%)	0.5
Median income of zip code	$53,952 ± 16,859	$53,480 ± 16281	$54,449 ± 17,123	0.7
Median income of zip code • 1st Quartile (lowest) • 2nd Quartile • 3rd Quartile • 4th Quartile (highest)	 91 (24.9%) 91 (24.9%) 89 (24.3%) 95 (26.0%)	 19 (24.4%) 18 (23.1%) 21 (26.9%) 20 (25.6%)	 68 (24.5%) 68 (24.5%) 68 (24.5%) 74 (26.6%)	1.0
Weekend collection** • Yes • No	 81 (21.5%) 295 (78.5%)	 20 (25.0%) 60 (75.0%)	 60 (21.0%) 226 (79.0%)	0.4

**Ten patients are missing collection information*

***Weekend collection includes Saturday and Sunday*

Summary

Introduction:

Approximately half of adult stone formers submit specimens that are either under- or over-collections as determined by 24-hour Creatinine/kg. Previously identified predictors of inadequate collection in adults include female sex, older age, higher body mass index (BMI), vitamin D supplementation and weekday collection.

Objective:

The objective of this study is to determine risk factors for inadequate 24-hour urinary specimen collection in the pediatric population.

Study Design:

We performed a retrospective analysis of all children (<18 years of age) with renal and/or ureteral calculi evaluated at our tertiary care pediatric center from 2005-2015. We included those who had at least one 24-hour urinary metabolic profile after a clinical visit for kidney and/or ureteral stones; we excluded children with bladder stones. Adequate collections had a urine creatinine of 10-15 mg/kg/24 hours. We performed a bivariate analysis of potential factors associated with inadequate collection of the initial urinary metabolic profile including child demographics, parental socioeconomic factors, history of stone surgery and weekday vs. weekend urine collection. We also performed a mixed effects logistic regression, controlling for correlation of specimens from the same patient, to determine whether an initial inadequate collection predicted a subsequent inadequate collection.

Results:

Of 367 patients, 80 had an adequate collection (21.9%): median age 13 years (interquartile range 8-16), 61.1% female, 93.5% white, 19.5% obese and 13.0% overweight. No parental or child factors were associated with inadequate collection (Summary Table). Of inadequate collections, over 80% were over-collections. In the 175 patients with more than one 24-hour urinary specimen collection, the effect of an initial inadequate collection on subsequent inadequate collections was not significant after controlling for the correlation of samples from the same patient (p=0.8).

Discussion:

We did not find any parental or child factors associated with the collection of inadequate 24-hour urine specimens in children. An initial inadequate collection does not predict subsequent inadequate collections. We were surprised that >80% of our inadequate collections were over-collections rather than under-collections. Possible explanations are that children collected urine samples for longer than the 24-hour period or that stone-forming children produce more creatinine per 24-hour period than healthy children due to hyperfiltration.

Conclusion:

Inadequate collections are very common and the risk factors for them are unclear. We would suggest a repeat collection if the first is inadequate. We plan further studies to explore barriers to accurate specimen collection using qualitative research methodology.

Introduction

Children with kidney stones and associated metabolic abnormalities have a high risk of recurrence.[1]Therefore, the 2016 American Urological Association guidelines recommend a complete metabolic evaluation in all first-time pediatric stone formers. [2] This evaluation

consists of the measurement of serum electrolyte levels in addition to a 24-hour collection of urine. The serum studies include calcium, magnesium, phosphorus and creatinine. The urinary studies assess the excretion of calcium, oxalate, uric acid, citrate, magnesium, phosphorus, sodium and potassium. In addition, the evaluation includes urine pH, volume, creatinine, sulfate, urea nitrogen levels and supersaturation. Providers determine the adequacy of this collection by calculating the ratio of creatinine to patient weight in kilograms adjusted by sex and age in children.[3]

Prior studies have identified two primary issues with the collection of a 24-hour urine specimen: a) non-completion and b) inadequate collection. Ellison et al noted that only 12% of children with incident stones completed a 24-hour urine collection within six months of their diagnosis.[4] Younger patients (6-12 years old) and those who underwent urologic or nephrologic evaluation were significantly more likely to submit the specimen compared to teenagers and those who did not have subspecialty evaluation.[4] Milose et al found that only 7.4% of adults at high risk for stone recurrence completed a 24-hour urine collection within six months of diagnosis.[5] Region of residence, type of comorbid illness and type of physician were significantly associated with completion.[5] Ghiraldi and colleagues found that 43% of adult stone patients living in an underserved, urban area submitted 24-hour urine specimens.[6] Caucasians and those with a family history of stone disease were more likely to submit a specimen than African-Americans and those without a family history.[6]

Several studies have demonstrated that approximately 50% of adult stone formers submit inadequate 24-hour urine specimens.[7, 8] Previously identified risk factors for inadequate specimen collection in adults include female gender and vitamin D supplementation.[7] McGuire et al found that the laboratory was 1.6 times more likely to receive adequate collections

on a Sunday and twice as likely to receive them from sedentary workers.[7] Patients with diabetes mellitus were 1.4 times more likely to submit adequate samples.[7] Sawyer et al found that under-collection was associated with increasing age and increases in body weight and body mass index.[8] They also noted that median calcium excretion increased significantly with increasing Cr/kg (i.e. "over-collection").[8] After a review of the literature, we were unable to identify **any** prior studies that determined predictors of inadequate specimen collection in pediatric patients. Therefore, the purpose of this study was to examine potential patient- and parent-level factors associated with inadequate collection of 24-**hour** urine specimens in a sample of pediatric patients with incident kidney stones.

Materials and Methods

We performed a retrospective analysis of all children (<18 years of age) with incident renal and/or ureteral calculi evaluated at our institution between 2005 and 2015. In order to build a comprehensive stone database, we performed a query of our billing system for all pediatric patients with a stone-related ICD-9 or ICD-10 diagnosis code associated with a clinical visit at our institution during the study period**. We included ICD-9 codes for kidney, ureteral and bladder stones. We also included ICD-10 codes for kidney, ureteral and bladder stones, urinary calculus unspecified, calculus in urethra, other lower urinary tract calculus and calculus of lower urinary tract unspecified.**

We also requested Litholink data for all pediatric patients from our institution during the study period. The billing data and Litholink data were then merged based on name and date of birth. We only included those who had at least one 24-hour urinary metabolic profile after a stone-related clinical visit. We excluded patients who had their first clinical visit prior to 2005

and patients who had a Litholink study performed prior to their first stone-related clinical visit. We also excluded children with only bladder stones.

Litholink distributed detailed, written instructions on proper 24-hour urine collection to all families prior to specimen collection. The handout, consisting of an illustrated 11-step process, instructs patients to flush their first morning urine in the toilet prior to starting the 24-hour urine collection. The instructions also state that patients should include the "very first urine the following morning" and then stop the collection. Litholink provided a standard container for urine specimen collection to all patients.

We extracted child demographics (age, race, sex, body mass index (BMI) percentile) and parental socioeconomic factors (insurance status and median income by Indiana zip code). We classified patients with 85th to <95th body mass index (BMI) percentile as overweight and those with ≥95th percentile BMI as obese.[9] **We included BMI percentile data because of the association between under-collection and increasing BMI and weight in adults.[8]** We obtained data on median income by Indiana zip code from the 2014 United States Census data[10] **We included income and insurance data as markers of socioeconomic status that are likely correlated with parental educational level.** We also determined whether the patient had a history of stone surgery and whether they performed weekend or weekday specimen collection. We evaluated the effects of a weekend urine collection due to possible impact of school attendance on the proper collection of the specimen. Adequate collections were defined by a urine creatinine of 10-15 mg/kg/24 hours in pre-pubertal patients defined as less than 16 years of age.[3] We used adult criteria (urine creatinine 15-20 mg/kg/24 hours) to define an "adequate collections in patients ages 16-17. **We estimated the glomerular filtration rate (GFR) using the updated bedside Schwartz formula: [0.413 * height (cm)]/serum creatinine**

(mg/dl).[11] Wedefined glomerular hyperfiltration as a GFR >/= 140 ml/min per 1.73m^2 and chronic kidney disease as a GFR <90 ml/min per 1.73m^2[11, 12]

We performed a bivariate analysis of potential factors associated with inadequate collection of the first urinary metabolic profile including child demographics, parental socioeconomic factors, history of stone surgery and weekday vs. weekend urine collection. We also performed a mixed effects logistic regression, controlling for correlation of specimens from the same patient, to determine whether an initial inadequate collection predicted subsequent inadequate collection. **We analyzed whether renal function was associated with collection adequacy on bivariate analysis in order to determine whether over collection could be due to hyperfiltration. We also examined the association between collection adequacy and 24-hour urine abnormalities on bivariate analysis. In addition, we performed a parallel analysis, re-classifying the "over-collections" (urine creatinine >15mg/kg/24 hours) as adequate collections in order to address the possibility that over-collections were erroneously labeled as inadequate. In this analysis, we classified "under-collections" (urine creatinine <10 mg/kg/24 hours) as inadequate.** The Institutional Review Board approved the study.

Results

Of the 376 children with incident kidney and/or ureteral stones and a 24-hour urine collection during the study period, **366/376 (97.3%) had complete collection information including a urine creatinine.** Of the 366 with complete information, 286/366 **(78.1%)** had an inadequate initial collection and 80/**366 (21.9%)** had an adequate initial collection. Of these, 248/286 (**86.7**%) were inadequate due to over-collection and 38/**286** (13.**3**%) were due to under-

collection. Mean age **of the entire cohort of 366 children** was **11.1 ± 3.9 years**, **54.8%** were female, **95%** were **Caucasian** and **65.2% had private insurance (Table 1)**. Patients with inadequate collections were similar in age to those whose collections were adequate (10.9 ± 3.8 versus 11.9 ± 4.2 years, p=0.1) (Table 1). No other parental or child factors were associated with inadequate collection. Of the 366 patients with 24-hour urine collections, 175 of them had ≥ 1 specimen collection. Approximately 80% of the second specimen collections were inadequate (Table 2). If the initial collection was **inadequate**, 100% of subsequent collections were also **inadequate. In contrast,** if the initial collection was **adequate**, 72% of subsequent collections were **inadequate** (p=0.01). Initial adequacy persists: all patients with initial inadequate collections had subsequent inadequate collections.

In the 175 patients who had repeated 24-hour urine collections, however, initial inadequate collection did **not** predict subsequent inadequate collections after controlling for the correlation of samples from the same patient (p=0.8). **This analysis examines the effect of an inadequate first test on any subsequent test and takes into account all of the tests a patient had.**

We also examined whether urine collection adequacy was associated with specific abnormalities on the 24-hour urine specimen (Table 3). We noted that over collections were more likely to have the following abnormalities than under collections or adequate collections: high calcium, low magnesium, low potassium, high sodium, low citrate, high pH and high phosphorus. Under collections and adequate collections were more likely to have low oxalate, low sodium and low phosphorus compared to over-collections. In the parallel analysis with over-collections re-classified as adequate, we found that patients with an inadequate collection were more likely to be female and have a higher BMI percentile

(Table 4). There was no significant difference in GFR between the collection groups (p=0.5) (Table 5).

Discussion

In this study, we found that approximately 80% of patients submitted inadequate 24-hour urine specimens that were mostly over collections. We did not identify any specific child or parental factors that predicted inadequate specimen collection. Previously identified risk factors in adults may be markers of immobility such as increased age and BMI and vitamin D supplementation. These risk factors may not be applicable to the majority of pediatric stone-formers. In contrast to the study by McGuire et al, we did not find a "weekend effect" of improved adequacy of specimen collection.[7] This is surprising given that weekday school schedules could theoretically interfere with adequate specimen collection in children.

We also found that an initial inadequate collection was not predictive of subsequent inadequate collections, taking into account all of the collections a patient performed **and accounting for correlation of specimens from the same patient.** Thus, we would suggest a repeat collection if the first is inadequate. The reasons for inadequate collection may overlap with reasons for non-completion of 24-hour urine collections. There is a high prevalence of non-completion of 24-hour urine collections in adult and pediatric stone-formers.[4, 5] Only 7.4% of adult stone formers at high risk for recurrence completed 24-hour urine testing within 6 months of diagnosis.[5] In the pediatric population, only 12% submitted a 24-hour urine collection within 6 months of diagnosis.[4] Completion of 24-hour urine collection was more common among younger patients and those who visited urologists or nephrologists.[4] We did not examine the effect of subspecialty visit on adequacy of collection since a urologist and/or

nephrologist evaluated all of our patients in our pediatric stone clinic. We also did not find any association between patient age and adequacy of collection.[4] In a study of adult stone-formers in an underserved area, Ghiraldi et al demonstrated that African-American patients were half as likely to submit an initial 24-hour urine specimen compared to Caucasian patients.[6] We did not find any association between race and adequacy of collection although our sample included very few non-Caucasian patients. Prior studies have also shown that patients may not receive adequate pre-collection instructions.[13] **Interestingly, when we re-classified over collections as adequate we found that patients with an inadequate collection were more likely to be female and have a higher BMI percentile. These findings are consistent with those of previous studies in the adult population.[7, 8]**

We were surprised that >80% of our inadequate collections were over collections rather than under collections. This is in contrast to the adult data which indicated that approximately three-fourths of samples were under-collections.[8] **High urine creatinine in the 24-hour urine specimen could be caused by one or more of the following factors: 1) over collection, 2) body composition differences, 3) tubular secretion of protein, 4) high protein diet or 5) hyperfiltration.** One explanation for over collections in children is that they collect urine samples for longer than the 24-hour period. In addition, t**he creatinine level in a 24-hour urine sample can be affected by several variables including muscle mass, renal function and protein intake.[14-16] Increased creatinine excretion into the urine masking over collection could theoretically be caused by increased muscle mass, increased protein intake and/or hyperfiltration. Conversely lower excretion of creatinine into the urine could be caused by low muscle mass, lower protein intake or decreased GFR. In our study, however, hyperfiltration was not associated with over collection of the 24-hour urine specimen. Of**

the patients with adequate collections, however, 8.8% of them were noted to have hyperfiltration which is a novel finding. The over collected specimens contained clinically relevant abnormalities whereas the under collected specimens did not. Therefore, over collections are more concerning than under collections because misinterpretation of the data from an over collected sample may lead to erroneous prescription of medications such as potassium citrate and/or diuretics to address hypocitraturia and/or hypercalciuria.

Future studies that compare healthy versus kidney stone patients and/or control for estimated GFR and collect 24-hour urine samples in a controlled inpatient setting will lend insight into whether our definitions of an adequate collection apply to the kidney stone population. If the inadequate collection is due **to** not all urines being collected or the time of collection not being a precise 24-hour period, the reasons for inadequate collection are likely multifactorial. The limitations of our study include its **retrospective nature and** small sample size with lacking racial and ethnic diversity. Our sample may lack the statistical power to determine an association between race/ethnicity and specimen adequacy. There may be other unmeasured socioeconomic factors that could impact the adequacy of specimen collection. Although we attempted to measure as many relevant demographic and socioeconomic factors as possible, the information available in the patients' medical records was a limitation of the study. Other potential factors may include the level of parental support in the home (e.g. single vs. dual parent household), parental work schedules, transportation issues and parental education level. We were not able to examine parental education level since this information is not typically available in the child's medical record. We attempted to use proxies for education level, however, such as median income by zip code and insurance status. **Finally, we do not know specifics of the verbal instructions the parents may have received about the proper**

technique of specimen collection. Future directions include a qualitative study to determine barriers to adequate specimen collection from the parental and child perspective. We hope to develop an intervention to address these barriers in order to improve the quality of care for children with stone disease.

Conclusions

The vast majority of patients submit an inadequate specimen for their initial 24-hour urine collection with over collection being more common than under collection. An initial inadequate collection does not predict subsequent inadequate collections. Thus, we would suggest a repeat collection if the first is inadequate. **It is often impossible to have a perfect 24-hour urine collection and therefore, more than one collection may be necessary to adequately evaluate a child's metabolic situation.**

Conflict of Interest

None

Ethical approval

Not required

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Table 2: Percentage of inadequate collections in initial and subsequent specimens

Inadequate collection #	**N (%)**
1st	287 (76.3%)
2nd	139 (79.4%)
3rd	87 (79.8%)
4th	52 (71.2%)
5th	40 (74.1%)

Table 3: Association of collection adequacy and 24-hour urine abnormalities

Abnormality	Under collection N=38 N (%)	Adequate N=80 N (%)	Over collection N=248 N (%)	p-value
High calcium	3 (7.9%)	18 (22.5%)	85 (34.3%)	**0.001**
Low calcium	0	0	0	-
High magnesium	3 (7.9%)	1 (1.3%)	9 (3.6%)	0.2
Low magnesium	26 (68.4%)	57 (71.3%)	221 (89.1%)	**<0.0001**
High oxalate	6 (15.8%)	12 (15.0%)	48 (19.4%)	0.6
Low oxalate	4 (10.5%)	5 (6.3%)	3 (1.2%)	**0.002**
High potassium	0	0	2 (0.8%)	1.0
Low potassium	27 (71.1%)	61 (76.3%)	223 (89.9%)	**0.0005**
High sodium	7 (18.4%)	20 (25.0%)	141 (56.9%)	**<0.0001**
Low sodium	1 (2.6%)	2 (2.5%)	0	**0.03**
High uric acid (no lower limit)	3 (7.9%)	2 (2.5%)	18 (7.3%)	0.3
Low citrate (no upper limit)	26 (68.4%)	55 (68.8%)	109 (44.0%)	**<0.0001**
High pH	14 (36.8%)	27 (33.8%)	40 (16.1%)	**0.0003**
Low pH	4 (10.5%)	12 (15.0%)	21 (8.5%)	0.2
High phosphorus	1 (2.6%)	7 (8.8%)	63 (25.4%)	**0.0001**
Low phosphorus	18 (47.4%)	14 (17.5%)	12 (4.8%)	**<0.0001**
*Hyperfiltration**	4 (14.3%)	7 (11.3%)	5 (2.7%)	**0.006**

**Based on data available from 275 patients (hyperfiltration defined as GFR ≥ 140)*

Table 4: Child demographic and parental socioeconomic characteristics of incident stone formers with 24-hour urine collections: over collections re-classified as adequate (n=366*)

Variable	Adequate Collection** (N=328)	Inadequate Collection (N=38)***	p-value
Race			
• African-American	6 (1.8%)	0 (0%)	0.4
• Caucasian	314 (95.7%)	36 (94.7%)	
• Other	3 (0.9%)	1 (2.6%)	
• Missing/Refused/Unknown	5 (1.5%)	1 (2.6%)	
Sex			
• Female	171 (52.1%)	28 (73.7%)	**0.01**
• Male	157 (47.9%)	10 (26.3%)	
Stone procedure			
• Yes	124 (37.8%)	12 (31.6%)	0.5
• No	204 (62.2%)	26 (68.4%)	
Age (years)	11.2 ± 3.6	10.7 ± 6.1	0.5
BMI Percentile	52.8 ± 35.0	66.5 ± 33.5	**0.03**
Insurance Category			
• Private	220 (67.1%)	21 (55.3%)	0.1
• Public	89 (27.1%)	16 (42.1%)	
• Self-Pay or Missing	19 (5.8%)	1 (2.6%)	
Median income of zip code	$54,451 ± 16,895	$52,388 ± 17,303	0.5
Median income of zip code			
• 1st Quartile (lowest)	76 (23.8%)	11 (29.7%)	0.4
• 2nd Quartile	79 (24.8%)	7 (18.9%)	
• 3rd Quartile	77 (24.1%)	12 (32.4%)	
• 4th Quartile (highest)	87 (27.3%)	7 (18.9%)	
Weekend collection****			
• Yes	72 (22.0%)	8 (21.1%)	0.9
• No	256 (78.0%)	30 (78.9%)	

**Ten patients are missing collection information*

*****Adequate collection includes patients with a urine creatinine ≥10 mg/kg/24 hours. This includes patients' collections previously classified as "over-collections" with a urine creatinine of >15 mg/kg/24 hours.***

****** Inadequate collection is defined as a urine creatinine <10 mg/kg/24 hours***

*****Weekend collection includes Saturday and Sunday*

Table 5: Association of estimated glomerular filtration rate and urine collection adequacy

GFR by Collection Group*	N	Mean	SD	Median	Lower Quartile	Upper Quartile	Minimum	Maximum
Under collection	28	118.1	51.2	105.8	85.1	125.7	72.0	309.5
Adequate	62	115.0	61.4	102.5	87.2	125.9	55.9	524.5
Over collection	185	108.7	96.8	100.7	88.3	116.3	56.6	1388.4

*p=0.5

Table 1: Child demographic and parental socioeconomic characteristics of incident stone formers with 24-hour urine collections (n=366)

Variable	Overall (N=366)	Adequate Collection (N=80*)	Inadequate Collection (N=286)	p-value
Race • African-American • Caucasian • Other • Missing/Refused/Unknown	 8 (2.1%) 357 (95.0%) 5 (1.3%) 6 (1.6%)	 1 (1.3%) 77 (96.3%) 2 (2.5%) 0 (0%)	 5 (1.7%) 273 (95.5%) 2 (0.7%) 6 (2.1%)	0.3
Sex • Female • Male	 206 (54.8%) 170 (45.2%)	 48 (60.0%) 32 (40.0%)	 151 (52.8%) 135 (47.2%)	0.3
Stone procedure • Yes • No	 139 (37.0%) 237 (63.0%)	 33 (41.3%) 47 (58.8%)	 103 (36.0%) 183 (64.0%)	0.4
Age (years)	11.1 ± 3.9	11.9 ± 4.2	10.9 ± 3.8	0.1
BMI Percentile	54.1 ± 35.0	51.8 ± 38.1	54.7 ± 34.2	0.5
Insurance Category • Private • Public • Self-Pay or Missing	 245 (65.2%) 111 (29.5%) 20 (5.3%)	 49 (61.3%) 25 (31.3%) 6 (7.5%)	 192 (67.1%) 80 (28.0%) 14 (4.9%)	0.5
Median income of zip code	$53,952 ± 16,859	$53,480 ± 16281	$54,449 ± 17,123	0.7
Median income of zip code • 1st Quartile (lowest) • 2nd Quartile • 3rd Quartile • 4th Quartile (highest)	 91 (24.9%) 91 (24.9%) 89 (24.3%) 95 (26.0%)	 19 (24.4%) 18 (23.1%) 21 (26.9%) 20 (25.6%)	 68 (24.5%) 68 (24.5%) 68 (24.5%) 74 (26.6%)	1.0
Weekend collection** • Yes • No	 81 (21.5%) 295 (78.5%)	 20 (25.0%) 60 (75.0%)	 60 (21.0%) 226 (79.0%)	0.4

**Ten patients are missing collection information*

***Weekend collection includes Saturday and Sunday*