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Factors Associated With Abnormal Initial 24-Hour Urine Studies in Pediatric Nephrolithiasis: Can We Better Select Patients for Evaluation?

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Background: Children, especially adolescents, constitute the most rapid growing demographic of nephrolithiasis. Due to the risks of recurrent stone disease, a 24-h urine analysis is recommended for the evaluation of children at risk of recurrent nephrolithiasis or those who are otherwise interested in further evaluation. However, data regarding patients most likely to have abnormal urine studies are sparse. We aim to identify predictors of abnormal 24-h urine studies in children presenting for evaluation of nephrolithiasis.

Methods: A retrospective review of children ≤17 years of age with a diagnosis of nephrolithiasis at both primary children's hospitals within our state from 2012 to 2017 was performed. Children with an adequate initial 24-h urine study (creatinine ≥9 mg/kg/24 h) not on a thiazide or potassium citrate during the study were included. Factors associated with any abnormality [calcium ≥4 mg/kg; oxalate ≥45 mg/1.73 m^2; citrate ≤310 mg/1.73 m^2 (girls) or ≤365 mg/1.73 m^2 (boys)] were evaluated as well as magnesium, uric acid, volume, sodium, and phosphorus.

Results: A total of 111 children were included, 69 of whom (62%) had at least one abnormal result. Of factors hypothesized to be associated with an abnormal 24-h urine study, only sex was significant ($p = 0.001$). Boys had a greater proportion of hypercalciuria (55%) and hypocitraturia (73%) and a slightly lower proportion of hyperoxaluria (48%) than those in girls.

Conclusion: Male sex was the only factor associated with an abnormal 24-h urine study, largely driven by increased rates of hypercalciuria and hypocitraturia in boys.

Keywords: pediatric, urolithiasis, risk factors, urinalysis, 24-hour urine analysis

INTRODUCTION

Pediatric nephrolithiasis has seen a steady rise in the past several decades, with adolescents showing the greatest rise in incidence across the entire age spectrum (1). These children have a 50% recurrence rate within 3 years, although risk stratification across the pediatric spectrum of disease has not been well defined (2). Each recurrent event poses additional risk for radiation exposure, anesthetic need, and surgical intervention, each of which carries unique morbidity in the pediatric population (3–6). Historically, there is limited research on the etiology of nephrolithiasis in pediatric patients.

Additionally, even in the absence of a known genetic cause of stones, nephrolithiasis may be a symptom of a more systemic disease process. Kidney stones may be one element of a larger disorder of calcium homeostasis. Preliminary work has suggested links between atherosclerosis or precursors to vascular disease in children with nephrolithiasis (7, 8). Furthermore, low bone density and an increased risk of skeletal fractures have also been associated with pediatric stone disease (7, 9, 10). Thus, evaluating the risk factors for urinary stone disease may have value in further understanding these more insidious disease processes and future health consequences in addition to assessing modifiable factors for risk reduction of future stone disease.

Traditionally, a 24-h urine analysis is recommended for the evaluation of patients at risk of recurrent nephrolithiasis, which includes all children diagnosed with stone disease (11). Children performing a 24-h urine analysis have been shown to have a 60% lower risk of stone recurrence, although the driving factors behind this association are unclear (2). While this evaluation can be beneficial in terms of highlighting underlying metabolic factors and guiding or reinforcing dietary strategies, they are expensive and logistically tasking on patients and their families (12, 13). Consequently, those who are economically disadvantaged are at risk of not completing the evaluation (12). The combination of these barriers has led to low completion rates and inadequate collections of 24-h urine in pediatric patients (12). Furthermore, even when a sample is adequate, a normal collection may not lead to a change in management (2, 13).

Due to the financial strain and the difficulty of obtaining a 24-h urine, the identification of patients most likely to have an abnormal urine study could avoid the unnecessary testing burden on patients and families. However, data regarding pre-evaluation factors indicative of a greater probability for 24-h urine abnormalities are sparse. To address this, we investigated patients with nephrolithiasis with 24-h urine studies in order to identify predictors of abnormal urine studies known prior to such an evaluation. These results could ultimately allow for a more effective selection of patients for urinary metabolic analysis. Because previous data have suggested that the highest rate of rise of kidney stone disease is in female subjects and adolescents, we hypothesized that female subjects and adolescents would be more likely to have abnormal 24-h urine studies.

METHODS

Patient Selection

We performed a retrospective chart review of children aged 0–17 years presenting with an index visit of nephrolithiasis at either of the two tertiary pediatric hospitals in the state, defined as the first visit for nephrolithiasis on or following January 1, 2012 (Site A), or January 1, 2014 (Site B) (based on the initiation of the electronic health record at each institution) and before January 1, 2018. The institutional review boards of each institution independently approved the study for research purposes. Given the minimal risk nature of this retrospective review, waivers of assent and consent were requested and granted by each institution. Children were included if they submitted a 24-h urine study for their evaluation during the study period. The decision to obtain a 24-h urine study was made through shared decision-making with providers and families. Children were excluded if they were on inhibitory medications for nephrolithiasis (i.e., thiazide, potassium citrate) or were lacking a height and weight at the time of the index visit (2). Additionally, 24-h urine samples were excluded if inadequately collected based on a primary and more restrictive definition, as outlined below.

Data Abstraction

The electronic health records including clinic visits, operative reports, lab results, and imaging were reviewed for data abstraction and entered into the Research Electronic Data Capture (REDCap) system housed at the data coordinating center (14). Patient demographics, comorbidities, personal and family history of nephrolithiasis, and 24-h urine results were analyzed. Comorbidities known or hypothesized to increase the risk of nephrolithiasis were captured including immobility, epilepsy, dependence on enteral feeding, neurogenic bladder, recurrent urinary tract infections (≥three infections per year documented preceding the index stone event), and prematurity. Medications known to or hypothesized to promote kidney stone formation (zonisamide, topiramate, furosemide, and prednisone) were recorded from the medication administration record at the time of the index clinic visit. Imaging review recorded the largest documented calculus. The follow-up interval was defined as the time to the most recent imaging follow-up.

24-Hour Urine Analysis

The need for a 24-h urine study was determined by the treating provider. All 24-h urine studies were performed *via* one of two centralized laboratories [Litholink Corp. (Chicago, IL, USA) and UroRisk, Quest Diagnostics (Secaucus, NJ, USA)]. Abnormal urine studies were defined *a priori* as follows: calcium >4 mg/kg; oxalate >45 mg/1.73 m^2; citrate <310 mg/1.73 m^2 (girls) or <365 mg/1.73 m^2 (boys) (15). Inadequate samples were defined as previously described by Saitz et al. (16) as ≤9 mg/kg/creatinine for our primary analysis. Acknowledging variable definitions for inadequate urine specimens, a sensitivity analysis used a more restrictive definition utilized by Litholink Inc., specifically, adequate creatinine/kg in boys of 11.9–24.4 and in girls of 8.7–20.3. If two consecutive 24-h

urine studies were received (i.e., 48-h urine collection), the results for each analyzed component were averaged over the time period and counted as one sample. *Post-hoc* analyses for additional 24-h urine findings [volume, supersaturation of calcium oxalate (SS CaOx), supersaturation of calcium phosphate (SS CaP), urinary magnesium, urinary phosphate, and urinary uric acid levels] evaluated these variables as continuous, as opposed to categorical variables.

Body Mass Index Percentile

Body mass index (BMI) was calculated in standard fashion from height and weight available within 6 months of the index visit. BMI and BMI-for-age percentile were determined using the publicly available calculator *via* the Centers for Disease Control (17). Obesity was defined as BMI percentile ≥85%.

Outcomes

Primary outcome was defined as any abnormality in the 24-h urine sample. Secondary outcomes were defined as the number and characterization of the specific 24-h urine abnormalities based on the abnormal definitions referenced above.

Secondary Analysis

Based upon the findings of our *a priori* hypothesis-driven analysis, we proceeded to perform several *post-hoc* analyses based on the primary finding of sex-associated differences in 24-h urine studies including comparisons of specific urinary citrate abnormalities by obese status and comparison of a more comprehensive set of urinary parameters as continuous variables.

Statistical Analysis

Descriptive and comparative statistics were performed using Stata v15.1 (College Station, TX, USA). Single or averaged consecutive samples were utilized, and no-repeat samples from patients were included to avoid clustering of data. Comparative statistics were performed between those children with normal and abnormal 24-h urine studies using an unpaired two-tailed t-test for continuous variables and a chi-square test for categorical variables. *A priori* hypothesized factors influencing 24-h urine findings were as follows: age (<12 years; ≥12 years); sex (male/female); race (white/all others); ethnicity (Hispanic or Latino/all others); comorbidity (present/absent); recurrent stone disease (present/absent); family history of stone disease (present/absent); obesity (18). As only sex was significant on univariate analysis, no multivariate regressions were performed. Statistical significance was defined as a p-value <0.05. Assessment of urinary parameter distribution for each variable as a continuous variable was performed using a test for skewness, revealing that none of these parameters were normally distributed. As such, descriptive statistics are reported using medians with interquartile ranges and comparative statistics by sex were performed using a Wilcoxon rank sum test.

RESULTS

Of the 127 children <18 years of age from both sites with a 24-h urine study, 16 were excluded for lack of weight or height data (4), inadequate collection (5), or on potassium citrate or thiazide medications (7). No children had a diagnosis of primary hyperoxaluria. The remaining 111 children were eligible for analysis. Our cohort ranged from ages 3 to 17 years. Among the 111 children, 69 (62.2%) had at least one urine abnormality. **Table 1** shows the demographic data of the included cohort. Thirty-eight (81%) boys had abnormalities in their 24-h urine studies, whereas 31 (48%) girls had abnormalities. Thirty-nine (74%) patients from Site A showed abnormal 24-h urine studies, whereas Site B had 30 patients (52%) with abnormalities, although this difference did not reach statistical significance. Comorbidities of patients with normal and abnormal 24-h urine studies are displayed in **Table 2**. Over half of the included population (60%) had one or more of the associated comorbidities. Substratified by comorbidity type, eight (100%) patients with immobility had abnormal 24-h urine studies. Nine children with gastrostomy tube (90%) had abnormal 24-h urine studies. Out of 10 children with epilepsy, seven (70%) of them had abnormal 24-h urine studies. Nine out of 11 children (82%) had abnormal 24-h urine studies while on a stone-promoting medication. **Table 3** displays stone characteristics and presenting symptoms in children with nephrolithiasis during the initial

TABLE 1 | Patient demographics, institution location, and history of nephrolithiasis of the included cohort.

	Normal	Abnormal	Total	p-value
Age (Mean, SD)	12.6 (4.4)	12.9 (4.1)	12.8 (4.2)	0.768
Age (N, %)				
Age ≤11 years	17 (41%)	25 (59%)	42	0.655
Age >12 years	25 (36%)	44 (64%)	69	
Sex (N, %)				
Boys	9 (19%)	38 (81%)	47	0.001
Girls	33 (52%)	31 (48%)	64	
Institution (N, %)				
Site A	14 (26%)	39 (74%)	53	0.018
Site B	28 (48%)	30 (52%)	58	
History (N, %)				
Personal history of stones	10 (45%)	12 (54%)	22	0.411
Family history of stones	27 (43%)	36 (57%)	63	0.212

SD, standard deviation.

TABLE 2 | Medical comorbidities of the included cohort stratified by any comorbidity and comorbidity by type.

	Normal	Abnormal	Total
Any comorbidity (N, %)	13 (40%)	19 (60%)	32
Comorbidity by type (N, %)[1]			
Neurogenic bladder	2 (25%)	6 (75%)	8
Recurrent urinary tract infection	5 (38%)	8 (62%)	13
Immobility	0 (0%)	8 (100%)	8
Gastrostomy tube	1 (10%)	9 (90%)	10
Prematurity			
Yes	5 (50%)	5 (50%)	10
No	20 (30%)	46 (70%)	66
Unknown	17 (49%)	18 (51%)	35
Epilepsy	3 (30%)	7 (70%)	10
Stone-promoting medication (N, %)[2]			
Medications	2 (18%)	9 (82%)	11

[1]"Comorbidity by type" will not add up to "Any comorbidity" total, as some patients had more than one comorbidity.
[2]Stone-promoting medications: zonisamide, topiramate, furosemide, and prednisone.

TABLE 3 | Presentation of nephrolithiasis symptoms in children with 24-h urine studies with associated imaging characteristics of their most recent kidney stone(s).

	Normal	Abnormal	Total
Presenting symptoms (N, %)[1]			
Pain	37 (43%)	49 (57%)	86
Obstruction	4 (33%)	8 (67%)	12
Infection	7 (70%)	3 (30%)	10
Other[2]	10 (33%)	20 (67%)	30
Incidental	4 (27%)	11 (73%)	15
Imaging characteristics (N, %)			
Stone size, Mean (SD)	5.4 (6.0)	6.4 (7.0)	6.0 (6.6)
Laterality			64
Unilateral	31 (37%)	53 (63%)	84
Bilateral	4 (36%)	7 (64%)	11

[1]Presenting symptoms may not add up to total cohort, as some children had more than one presenting symptom.
[2]Other symptoms included hematuria (N = 20) and/or nausea and emesis (N = 15); other symptoms may not add up to the total "Other" symptoms, as some children had more than one "Other" presenting symptom.

evaluation. Eight children (67%) with obstruction had abnormal 24-h urine studies. Out of 15 asymptomatic children, 11 (73%) had abnormal 24-h urine studies. There were no significant differences in unilateral stone location vs. bilateral stone location for abnormal 24-h urine studies, with 53 children (63%) with unilateral and seven (64%) with bilateral kidney stones. **Table 4** displays associating factors in abnormal 24-h urine studies. Thirty-eight male children (81%, $p < 0.001$) had abnormal 24-h urine results as compared to 31 (48%) female patients with abnormal 24-h urine studies. The remaining characteristics showed no statistical differences between normal and abnormal 24-h urine studies. **Figure 1** displays the specific 24-h urine abnormalities based on sex. There was no difference in results after an additional subanalysis of all factors performed with the exclusion of patients with known comorbid factors. **Table 5** displays the specific urinary parameter differences between male and female sex. There was a statistical difference in citrate and oxalate across sexes but no statistically significant differences in the comparison to other urinary parameters stratified by sex. Additional analysis showed no statistical difference between BMI and citrate levels (data not shown). Median 24-h urine volume/kg was stratified by any metabolite abnormality (14.5 ml/kg) vs. no abnormalities (8.6 ml/kg) ($p = 0.0318$, Wilcoxon rank sum test).

A subanalysis of 63 individuals who met more stringent criteria for 24-h urine collection adequacy found that sex-based differences in abnormal 24-h urine parameters remained significant between boys and girls (78% vs. 42%, $p = 0.004$), while other comparisons remained nonsignificant (data not shown).

DISCUSSION

This study analyzed initial 24-h urine studies of 111 eligible children with nephrolithiasis from both tertiary care pediatric hospitals in Wisconsin. Evaluation with a 24-h urine analysis is recommended by the American Urological Association (AUA) for all high-risk and interested patients, and completion of this test has been associated with a reduction in the recurrence of nephrolithiasis in pediatric patients (19). Indeed, Tasian et al. (2) reported a 60% risk reduction associated with the completion of 24-h urine studies in children and adolescents. Nonetheless, 24-h urine assessments are costly and tedious for patients to complete

TABLE 4 | Factors associated with abnormal 24-h urine studies. Obesity is defined as BMI >85%.

	Normal	Abnormal	Total	p-value
Age (Mean, SD)	12.6 (4.4)	12.9 (4.1)	12.8 (4.2)	0.7677
Sex (N, %)*				
Boys	9 (19%)	38 (81%)	47	0.0001
Girls	33 (52%)	31 (48%)	64	
Race (N, %)				
White	38 (38%)	62 (62%)	100	0.915
All others	4 (36%)	7 (63%)	11	
Ethnicity (N, %)				
Hispanic/Latino	3 (38%)	5 (62%)	8	0.984
All others	39 (38%)	64 (62%)	103	
Comorbidity (N, %)				
Any	13 (41%)	19 (59%)	32	0.700
None	29 (37%)	50 (63%)	79	
Obese				
Yes	15 (44%)	19 (56%)	34	0.365
No	27 (35%)	50 (65%)	77	
Recurrent nephrolithiasis (N, %)				
Yes	10 (45%)	12 (54%)	22	0.411
No	32 (36%)	57 (64%)	89	
Family history of stones (N, %)				
Yes	27 (43%)	36 (57%)	63	0.212
No/Unknown	15 (31%)	33 (69%)	48	
Presenting symptoms (N, %)				
Any	40 (41%)	58 (59%)	98	0.0076

**Significant difference in abnormal 24-h urine studies.*
SD, standard deviation; BMI, body mass index.

(12, 13). The tension between the potential benefit of these evaluations weighed against the burden of 24-h urine collections emphasizes the importance to risk-stratify children who may benefit from this testing. Chan et al. (20) found that a limited 24-h urine metabolic evaluation consisting only of urinary oxalate, calcium, citrate, and urine volume combined with a stone analysis would detect many significant metabolic abnormalities in the pediatric population. While a limited urinary metabolic evaluation is lower in cost, the additional data supplied with a complete 24-h urine collection (such as urinary salt supersaturation and urinary measurements of dietary factors) are critical in determining what further evaluation is necessary and often assist in devising a treatment strategy. Additionally, such an approach still does not eliminate

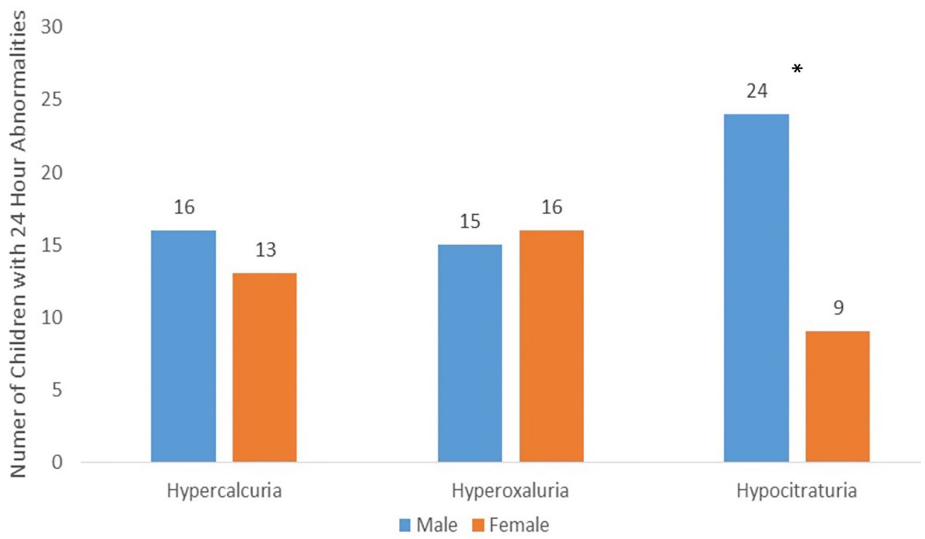

FIGURE 1 | The 24-Hour Urine Abnormalities: The three most common abnormalities in 24-h urine results stratified by sex. Hypocitraturia was found to be significantly higher ($p < 0.001$) in boys than that in girls. There were no significant differences in 24-h urine abnormalities by sex for hypercalciuria and hyperoxaluria.

TABLE 5 | The 24-h Litholink urinary parameters by sex.

Variable	Total Median (IQR)	Boys Median (IQR)	Girls Median (IQR)	p-value
Calcium/kg	3.0 (1.9–4.1)	3.1 (1.5–4.6)	3.0 (2.0–3.7)	0.616
Oxalate/BSA*	33.5 (25.3–45.3)	38.0 (26.5–50.3)	31.8 (24.7–41.2)	0.034
Citrate/BSA*	453.1 (290.3–684.7)	361.8 (200.2–593.8)	497.8 (394.2–728.9)	0.001
Uric Acid	0.4 (0.3–0.5)	0.4 (0.3–0.6)	0.4(0.3–0.5)	0.689
Volume/kg	12.1 (6.3–24.4)	14.7 (5.9–30.9)	10.0(6.3–21.2)	0.156
SS CaOx	8.4 (5.3–10.7)	8.5 (5.1–11.2)	8.2(5.4–10.4)	0.858
SS CaP	1.9 (1.2–3.1)	1.8 (1.3–2.7)	2.2 (1.2–3.3)	0.315
Magnesium	84.0 (56.0–114.3)	88.4 (58.0–143.0)	83.1 (55.5–101.8)	0.091
Phosphorus	0.6 (0.4–0.8)	0.7 (0.4–0.9)	0.6 (0.4–0.8)	0.657
Sodium	126.0 (76.0–160.7)	117.2 (76.0–173.4)	126.9 (77.0–156.5)	0.931

**Significant difference across sexes.*

BSA, body surface area; IQR, interquartile range; SS CaOx, supersaturation of calcium oxalate; SS CaP, supersaturation of calcium phosphate.

the need for a 24-h urine collection. For these reasons, the concept of a limited 24-h urine evaluation has not been widely adopted.

Improving the identification of clinical (i.e., prior to testing) factors associated with 24-h urine anomalies could help to target pediatric patients who clearly require further metabolic evaluations and therefore avoid requiring select children to perform the cumbersome 24-h urine collection process altogether. Because previous data have shown rising incidence rates of kidney stone diseases in female subjects and adolescents, we hypothesized that these groups would be more likely to have an abnormal 24-h urine evaluation and therefore more expansive metabolic evaluations could be targeted to these groups (1, 21). Our cohort is demographically representative of other reported studies on pediatric nephrolithiasis in terms of both female predilection and the proportion of adolescents represented. Surprisingly, of the pre-evaluation identifiers we studied, male sex was the only factor associated with abnormal 24-h urine analysis. As such, our study emphasizes the importance of considering sex-based differences in kidney stone etiology. Additionally, it strongly suggests that all children, including boys, presenting with nephrolithiasis should undergo a 24-h urine collection to evaluate metabolic abnormalities, as we did not identify a distinct pattern predictive of abnormalities beyond male sex.

When a 24-h urine analysis is used in the initial evaluation of pediatric patients, the presence of a metabolic risk factor on a 24-h urine evaluation is seen in as many as 69%–90% of pediatric patients (22, 23). Prior studies have demonstrated that the most common abnormalities in the pediatric population are hypercalciuria and hypocitraturia in pediatrics (22–25). These findings are consistent with our study's results with hypocitraturia (73% of boys, 27% of girls) and hypercalciuria (55% of boys, 44% of girls) as the most common abnormalities in our cohort. Hypocitraturia was the largest driver of differences in 24-h urine abnormalities across sexes.

Other studies have also identified sex-based differences in nephrolithiasis among various ages, although a specific focus on urinary abnormalities has been limited (26). Specifically, boys tend to have nephrolithiasis more commonly in the first decade of life, while girls more commonly exhibit nephrolithiasis in the second decade of life (27). Adolescent female subjects, in particular, have recently been identified as having the greatest increase in the incidence of nephrolithiasis across the entire life span of the disease (1). The variation in incidence rates seen across sexes may indicate a metabolic difference between male subjects and female subjects that contributes to previously reported differences in stone composition and urine studies (26). Female subjects tend to have struvite and calcium phosphate stones more commonly (28, 29). Healthy women have also been shown to have lower urinary calcium and higher citrate compared to men, which are likely protective factors against the formation of common calcium oxalate stones (30). Indeed, Kirejczyk et al. (31) found that healthy girls have been shown to have a large spike in urinary citrate when they reach adolescence. In the context of our findings, we believe that there may be an underappreciation of the protective effect of this urinary citrate surge, *vis-à-vis* typically reported "normative" values of citrate in adolescent female subjects. Hypocitraturia has also been found to correlate to lower consumption of potassium and magnesium suggestive of a possible dietary component to this change in urinary abnormalities. However, the specific drivers for alterations in urinary citrate levels as children age have not been well defined. Our data derive from a region where Western diets heavy on animal proteins, dairy, and processed food are prevalent, although it should be noted that we did not evaluate dietary parameters in our study specifically. Components of these diets have been linked to childhood obesity and may contribute to the risk of nephrolithiasis in children by altering the acid–base status of the urine, thereby reducing levels of buffered urinary citrate (32). Indeed, animal models focused on the specific impact of fructose consumption on urinary parameters demonstrated a decrease in inhibitors of urinary stone formation (33).

Although 24-h urine parameters are often evaluated in a dichotomized "normal" vs. "abnormal" fashion in clinical care, we acknowledge that risk assessment of urinary stone parameters exists on a continuum. As such, our secondary analysis evaluated urinary parameters as a continuous variable and stratified these by sex. Based on our findings, urinary citrate (higher in girls) and urinary oxalate (higher in boys) differed significantly across sexes. Urinary citrate is known to differ in adolescent male

subjects and adolescent female subjects, which was taken into account when we generated our definitions of hypocitraturia for this analysis (31). However, urinary oxalate has not been previously reported to differ in the pediatric population. Otto et al. (34) reported on differences in urinary oxalate excretion in adults, with men having a higher proportion of hyperoxaluria in their series. While our dichotomized analysis showed no difference in hyperoxaluria, evaluating oxalate as a continuous risk factor certainly raises the opportunity to further investigate sex-related differences in oxalate homeostasis in the pediatric population as well.

Dietary modifications and fluid intake are mainstays in secondary kidney stone prevention for both children and adults. Fluid intake goals are directed by the Institute of Medicine recommendations, although children with nephrolithiasis likely require augmented fluid intake beyond these recommendations (35). While data suggest that pediatric and adolescent male subjects may be more likely to reach their fluid intake goals within the healthy population, the relationship of these data to the kidney stone-forming population is unknown (36). Our data suggest no significant difference in weight-adjusted fluid intake as stratified by sex and support a broad-based approach to fluid intake assessments. Acknowledging the importance of fluid intake (and therefore volume) on urinary stone risk, we assessed 24-h urine volume/kg as a function of normal vs. abnormal parameters and found that those children with a documented 24-h urine metabolic abnormality had higher urinary volumetric outputs than those with no known abnormality. This finding may suggest that for those children with no defined metabolic abnormality, fluid intake is a greater driver of risk than for those children who have a defined metabolic abnormality. However, we would emphasize that management strategies regardless of the 24-h urine study findings should include fluid intake as a mainstay of prevention.

Strategies to improve fluid intake are of great interest to children with urinary stone disease, their families, and their providers. As such, the results of trials such as the Prevention of Urinary Stones with Hydration study may illuminate successful strategies in further detail and specifically have enrolled an adolescent subcohort to focus on the pediatric population (37). Dietary modifications may influence a number of urinary stone parameters. High dietary protein intake may impact urinary pH, uric acid, and citrate excretion, while a diet high in sodium may influence urinary magnesium and calcium homeostasis (38). Although sex-related differences in diet may exist, some data suggest that dietary changes are more age-related, with both peripubertal boys and girls eating greater amounts of fast food, which could influence both dietary protein and sodium intake (39). Our clinics do not routinely recommend limiting protein intake in children, given the low rates of uric acid-based nephrolithiasis in children and the need for dietary protein in longitudinal growth and development throughout childhood. However, dietary salt limitation is a routine focus of our clinics' standard dietary recommendations (15). While our study found no differences in assessment of unadjusted urinary magnesium, phosphorus, or uric acid across sexes, given the current paucity of data surrounding the importance of these factors in assessing urinary stone risk in children, further study is still needed. Unfortunately, we did not have standard dietary assessment documentation available to correlate these factors with dietary intake, although this focus would be ripe for future studies.

Our study has several limitations including its retrospective nature. Additionally, our institutions are tertiary care centers with a large referral population that may represent children more likely to have a 24-h urine evaluation recommended in their workup. Prior studies have also identified specific barriers to 24-h urine study completion, such as socioeconomic status and insurance status (12, 13). However, our study was unable to evaluate those children for whom a 24-h urine study was recommended but not completed. Furthermore, while including patients from multiple institutions expands the scope of representative patients, there should be caution in the generalizability of our results to patients who do not consume a western diet. The pediatric population also poses a challenge for the determination of adequate 24-h urine samples with urinary creatinine excretion varying significantly in pediatrics compared to adults due to their lower muscle mass, especially in those children with chronic disease states and muscle wasting. Importantly, our results were still significant after initial criteria were instituted and maintained even after subsequent analysis with a more stringent definition for adequate collections. Additionally, our study used only calcium, oxalate, and citrate to indicate an abnormal 24-h urine in a dichotomized fashion. Although there is a concern for possible missed abnormal 24-h urines, Chan et al. (20) demonstrated a limited urinary metabolic evaluation still detects the vast majority of clinically significant abnormalities. Lastly, we acknowledge that the 24-h urine analysis is not truly a dichotomized study, and the variation seen between urinary studies is quite significant. As such, further secondary analysis on a broader range of 24-h urine parameters was also performed. Additionally, the choice to act on these results is provider-dependent and can impact the usability of these data in clinical practice. However, the potential benefits of a 24-h urine evaluation due to its association with a 60% decreased risk of recurrence for pediatric nephrolithiasis cannot be ignored (2). While we cannot determine the clinical implications of our results, we believe that the significance of exploring sex-based differences is important in understanding the epidemiology of stone disease and the usefulness of 24-h urine evaluations going forward.

Despite these limitations, our multi-institutional study provides a large cohort to study 24-h urine analysis across pediatric stone formers. Our study sought to identify critical pre-evaluation factors to better select participants for 24-h urine screening, which can be tedious and costly, thereby maximizing their diagnostic potential. We discovered that male sex was the only pre-evaluation factor associated with an abnormal 24-h urine evaluation.

Recent studies have shown the relationship between sex and stone disease changes throughout the life span. This shift to assessing sex-based differences in kidney stone disease and our

own study's results add to the growing literature of biologic differences between sexes and propose the need for further evaluation of sex-based differences among pediatric stone formers. Our study also emphasizes the need for further investigation to delineate the value of 24-h urine evaluations and the possible benefits of risk stratification based on either 24-h urine anomalies or the risk of recurrent disease.

Our data also suggest that certain at-risk populations, such as children with epilepsy, gastrostomy feedings, or immobility, have a very high likelihood of having metabolic abnormalities on 24-h urine stone risk analysis, albeit underpowered in our analysis to detect a difference. Obtaining a 24-h urine collection from these patients is traditionally very logistically challenging; however, given the increased risk of identifying a potentially treatable urinary stone risk factor, it is important to pursue 24-h urine testing whenever feasible.

In conclusion, this study showed that male sex was significantly associated with an increased risk of an abnormal 24-h urine evaluation. Our study highlights the importance of evaluating sex-based differences in stone disease, especially in the pediatric population. With rising incidence rates and increased risk of recurrence in pediatric patients, further understanding of the pediatric stone disease is crucial in developing management strategies. Our data were unable to isolate other patient characteristics that could safely identify those patients who could avoid undergoing a 24-h urine metabolic workup. This finding reaffirms the current AUA recommendation that all patients at risk for future stone events, which at present time would include all children who present with nephrolithiasis, be offered a 24-h urine assessment to identify lithogenic risk factors. Future studies delineating sex-based differences will allow for a better understanding of 24-h urine evaluations and lead to a standardization of evaluation of pediatric stone formers, decreasing the use of unnecessary diagnostic evaluations in pediatric patients and overall care of pediatric stone formers.

DATA AVAILABILITY STATEMENT

De-identified raw data will be made available upon request, pending institutional data use agreement and regulatory review. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Children's Wisconsin IRB and American Family Children's Hospital IRB. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

The authors confirm contribution to the paper as follows: study conception and design: JE, NP data collection: AM, RM Author; analysis and interpretation of results: JE, NP, AM draft manuscript preparation: AM, JE, NP, KS All authors reviewed the results and approved the final version of the manuscript.

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