GENDER CHARACTERISTICS OF URINARY EXCRETION OF OXALATES, CALCIUM AND PHOSPHATES IN CHILDREN AND TEENAGERS

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Abstract. Relevance. The risk factors for urolithiasis include an increase in the daily excretion of lithogenic metabolites. These factors have not been sufficiently studied in childhood. Methods. We conducted a study of the 24-hour urinary excretion of calcium, oxalate and phosphorus in 196 patients (median age was 9.0 [6.0; 14.0] years, boys 23.5% (46/196)) with the urinary syndrome. Results. We found a statistically significantly higher daily urinary excretion of oxalates in boys compared to girls, 18.1 [11.2; 25.6] mg/day and 14.1 [9.6; 21.3] mg/day, respectively, p = = 0.012. However, these differences manifest themselves only at the age of 10 years and older, amounting to 23.85 [11.2; 25.5] mg/day in boys and 13.91 [8.02; 18.9] mg/day in girls, p = 0.005. We did not establish gender differences in daily calcium excretion, p = 0.45. At the same time, we revealed gender differences in daily phosphorus excretion in boys compared with girls 23.25 [15.0; 38.0] mmol/day and 18.9 [10.6; 29.2] mmol/day, respectively, p = 0.013. These patterns were also typical only for the age older than 10 years – in boys 31.7 [21.1; 43.0] mmol/day, in girls 17.9 [11.6; 30.9] mmol/day, p = 0.003. Conclusions. Boys aged 10 years and older have a statistically significantly higher 24-hour urinary excretion of oxalate and phosphorus than girls. This may indicate the gender dependence of some lithogenic factors and the need for increased attention to the prevention of nephrolithiasis during early puberty.

Keywords: lithogenic risk factors, metabolic nephropathy, gender differences.

Introduction

Urolithiasis has been described in medical reports from different countries and cultures for thousands of years, and it remains a common and recurrent disease, causing a significant global burden of dis-ease (Lopez & Hoppe, 2010). In the general population, the maximum frequency of urolithiasis falls on middle-aged, most working-age adults. In children, urolithiasis presents at any age. There are no reliable and complete data on the prevalence of urolithiasis among children and teenagers, however, in the last 10–15 years, an increase in the incidence of urolithiasis and nephrolithiasis has been noted (Tasian et al., 2016; Jobs *et al.*, 2021).

In the structure of urinary stone disease, in young adults, the formation of calcium oxalate and calcium-phosphorus calculi is of particular importance (Scales *et al*., 2016). A study by Israeli specialists has shown that in adults, the incidence of calcium oxalate dehydrate stone formation decreases with age (Usman et al., 2013).

Until now, there is no single concept of the pathogenesis of stone formation. It is generally accepted to consider urolithiasis a polyethiologic disease. One of the causes of stone formation is the formation of a supersaturated solution, and its most important predictors are the volume of urine and the concentration of lithogenic substances in the urine. However, at present, this mechanism is considered as the main one for the formation of cystine, xanthine and urate calculi, although according to the literature, the role of this mechanism in the formation of calcium oxalate nephrolithiasis is shown (Borghi *et al*., 1996; Lieske *et al*., 2010; Ogawa *et al*., 2003; Parks *et al*., 1997).

The crystals that have appeared serve as a focus for further growth of the stone (Bergsland *et al.*, 2012). In calcium nephrolithiasis, Randall's plaques are considered to be the initiators of stone formation (Mager & Neisius, 2019). Deposits leading to plaque formation develop on the basement membrane of the ascending

loop of Henle and spread into the interstitial space as size increases. Khan (Khan *et al*., 2021) suggest that urinary oversaturation contributes to kidney damage by inducing reactive oxygen species production and oxidative stress, and that the subsequent inflammatory immune response promotes Randall's plaque and calcium stone formation.

Thus, urinary oxalate, calcium and phosphate concentrations among children and adolescents are important predictors of urolithiasis in young adults. So, when analysing large epidemiological cohorts, Taylor and his colleagues (Taylor & Curhan, 2007) showed that urinary excretion of oxalate positively correlates with the risk of developing kidney stones. According to Jobs (Jobs *et al*., 2021) in every case of urolithiasis in the paediatric population, a metabolic assessment is required, since metabolic abnormalities can be found in most cases in this age group.

However, previous studies have yielded conflicting results regarding gender effects on lithogenic risk factors. In a group of 48 men from India with calcium nephrolithiasis, daily urinary oxalate concentration weakly correlated with free testosterone in serum (Pearson's coefficient = 0.297, *p*-value = 0.04) (Nath *et al*., 2013). In a study by Fuster and co-authors (Fuster *et al*., 2020), women were found to have significantly lower excretion of both calcium and oxalates compared to men.

Sex differences in nephrolithiasis in terms of both the prevalence and composition of stones can be explained by different interactions of genetic factors and environmental factors (Lieske *et al*., 2013; Miller, 2017; Otto *et al*., 2017; Perinpam *et al*., 2016), however, genetic risk factors may be less important for women than for men, as recently it has been shown in a study of twins (Goldfarb *et al*., 2019).

Thus, the clarification of the gender characteristics of crystalluria in childhood and adolescence is very important for the formation of metaphylaxis programs for urolithiasis in young adults.

Purpose of this study: To investigate gender differences in urinary excretion of calcium and oxalate in children and adolescents with urinary syndrome.

Materials and methods

Design study

The current study was a pilot, non-randomized, single-center, open-label, cohort study.

Patient characteristics

We checked 196 children and adolescents with urinary syndrome of the Department of Nephrology of the Children's Clinical Hospital No. 1 of Nizhny Novgorod at the age from 3 to 17 years, the median age was 9.0 [6.0; 14.0] years, boys 24.4 (48/196)%. The study was retrospective and did not require ethics committee approval. At the same time, in accordance with the existing legislation, measures were taken to anonymize the study. The examination and treatment of children was carried out in accordance with accepted standards and clinical guidelines according to the nosological diagnosis. The study did not include children with hereditary tubulopathies and stage 3–5 chronic kidney disease.

Research was retrospective and did not require ethics committee approval.

The collection of daily urine was carried out as directed by the attending physician in accordance with regulatory document (R53079.4-2008), while the daily urine volume was estimated.

Laboratory research methods. Determination of the content of oxalates, calcium, phosphorus oxalates and creatinine was carried out using standard biochemical laboratory methods in samples obtained on the basis of 24-hour urine collection.

Statistical analysis was performed using Statgraphics Centurion, v. 16.1.17. For quantitative traits, in order to determine the normality of the sample, standardized skewness and standardized kurtosis were calculated. If the calculated values of these indicators were outside the range –2… +2, then the con-sidered quantitative samples were considered different from normal. Thus, this sample is different from the normal one; later, nonparametric tests were used to determine the differences between the groups. Differences between the two groups were determined using the Wilcoxon W-test – a compari-son of the medians of the two samples.

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Table 1

Clinical characteristics of patients and the content of crystal-forming substances

Parameters	**All**	**Boys**	**Girls**	**Differences between groups**
Number of patients	196	46	150	–
	9.0 [6.0; 14.0]	10.5 [5.0; 15.0]	9,0 [6.0; 14.0]	W = 3384.5 p = 0.9
Oxalates, mg/day	14.9[10.0; 21.7]	18.1 [11.2; 25.6]	14,1 [9,6; 21,3]	W = 2600.5 p = 0.012
Calcium, mmol/day	1.30 [0.90; 2.00]	1.25 [0.90; 2.90]	1.30 [0.92; 1.80]	W = 3211.5 p = 0.45
Phosphates, mmol/day	19.9 [11.4; 30.4]	23.25 [15.0; 38.0]	18.9 [10.6; 29.2]	W = 2612.0 p = 0,013
pH	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	W = 3924.5 p = 0.12
Age under 10 years				
Number of patients	100	22	78	–
Oxalates, mg/day	18.4 [11.0; 24.7]	20.5 [13.6; 25.6]	17.7 [10,9; 24,7]	W = 737.0 p = 0.31
Calcium, mmol/day	1.57 [0.85; 1.86]	1.64 [0.8; 1.92]	1.55 [0.9; 1.60]	W = 909.5 p = 0.67
Phosphates, mmol/day	20.7 [10.7; 29.95]	21.67 [12.7; 26.5]	20,4 [10.2; 24.7]	W = 803,5 p = 0.65
pH	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	W = 964.5 p = 0.39
Age after 10 years				
Number of patients	96	24	72	–
Oxalates, mg/day	16.4 [9.44; 20.8]	23.85 [11.2; 25.5]	13.91 [8.02; 18.9]	W = 519.0 p = 0.0054
Calcium, mmol/day	2.00 [1.00; 2.30]	2.74 [1.01; 3.86]	1.75 [1.0; 1.90]	W = 670.5 p = 0.14
Phosphates, mmol/day	22.3 [12.1; 34.2]	31.7 [21.1; 43.0]	17,9 [11.6; 30.9]	W = 497.0 p = 0.003
pH	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	7.0 [6.0; 7.0]	W = 989,0 p = 0,21

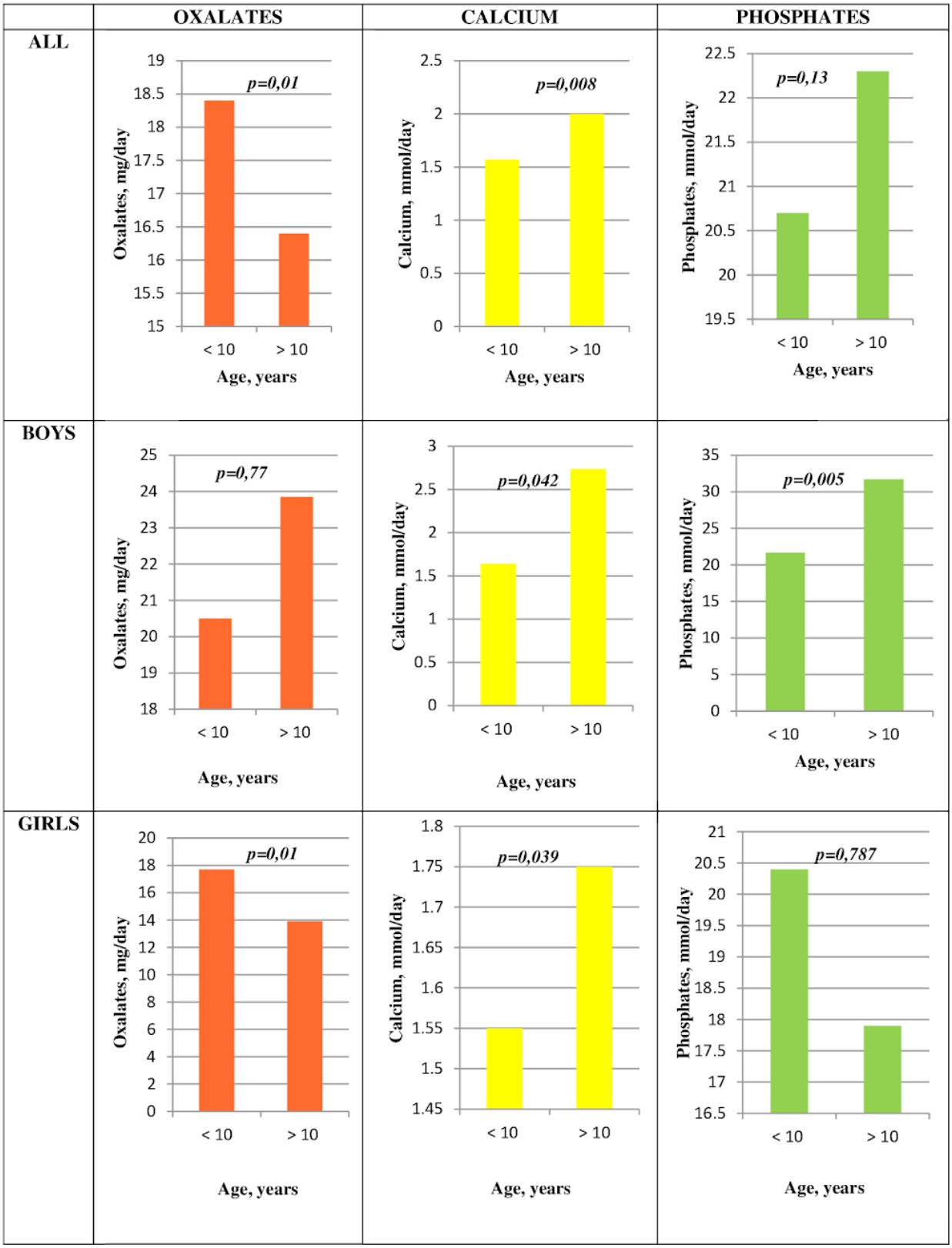

Fig. 1. Dependence of excretion of lithogenic risk factors on the age of patients (prepubertal (< 10 years) vs pubertal (>10 years))

Data are presented as Me [Q1; Q3], where Me is the median, [Q1; Q3] – the values of the first and third quartiles. To compare the medians of several groups, the Kruskal – Wallis test (KW test) was used. The relationship between parameters was assessed using Spearman's rank correlation (p). Dif-ferences between qualitative characteristics were analyzed using the $\chi 2$ test. Differences were consid-ered statistically significant at $p < 0.05$.

Results

The clinical characteristics of the patients and the content of crystal-forming substances are presented in Table 1. Girls and boys were comparable in age ($p = 0.9$).

We have identified that, in general, there is a statistically significantly higher daily urinary excretion of oxalates in boys compared to girls, 18.1 [11.2; 25.6] mg/day and 14.1 [9.6; 21.3] mg/day, respectively, $p = 0.012$. However, these differences manifest themselves only at the age of 10 years and older, in fact, at the onset of puberty, amounting to 23.85 [11.2; 25.5] mg/day in boys and 13.91 [8.02; 18.9] mg/day in girls, p = 0.0054.

It should be noted that the current standards for the excretion of oxalates in children are guided only by the weight / surface area of the child's body and do not have sexual differentiation (Bouzidi et al., 2016; Milliner et al., 2020).

We haven't identified that statistically significant gender differences in daily calcium excretion, p = 0.45. At the same time, we have revealed statistically significant gender differences in daily phosphorus excretion, which are higher in boys than in girls: 23.25 [15.0; 38.0] mmol/day and 18.9 [10.6; 29.2] mmol/day, respectively, $p = 0.013$. These patterns were characteristic only for the age of 10 years and older – in boys 31.7 [21.1; 43.0] mmol/day, in girls 17.9 [11.6; 30.9] mmol/day, $p = 0.003$.

Urine pH was comparable in boys and girls at all ages.

We revealed a statistically significant negative correlation between the daily excretion of oxalates and the age of female patients ($r = -0.21$, $p = 0.01$); in boys, no relationship was found. Also, a statistically significant positive correlation was found between calcium excretion and age, both in the general cohort ($r = 0.22$, $p = 0.002$) and separately in boys ($r = 0.33$, $p = 0.02$) and girls ($r = 0.17$, $p = 0.04$). We found a statistically significant correlation between phosphaturia and age in boys ($r = 0.37$, $p = 0.01$).

Also we analysed excretion of lithogenic risk factors in group before 10 years (usually prepubertal age) and after 10 age (usually puberty age), Figure 1.

You can see that daily excretion of all factors (oxalates, calcium, phosphates) higher in boy older 10 years (for calcium and phosphates difference were statistically significant, $p = 0.042$ and $p = 0.005$). In opposite in girls daily excretion of oxalates (difference were statistically significant, $p = 0.01$) and phosphates decreased in puberty.

Discussion

We did not find an associate between the daily urinary oxalate content and age in boys ($p = 0.67$), but we found a statistically significant decrease in oxaluria in girls as they grow older. We can therefore assume that the formation of reproductive endocrine regulation in girls is associated with a decrease in their daily secretion of oxalates and, possibly, with a decrease in the risk of calcium oxalate urolithiasis, which requires additional study. A possible reason is also a change in the nature of the diet of adolescents with a large consumption of meat products by male adolescents. Thus, Dursun (Dursun *et al*., 2016) found that urinary oxalate excretion positively correlated with increased protein intake and negatively correlated with age. The Clifford-Mobley (Clifford-Mobley *et al*., 2015) study found a lower daily excretion of oxalate in adult women compared to men. At the same time, the Novak (Novak *et al*., 2009) study showed a high incidence of urolithiasis in girls in the second decade of life.

We have not found statistically significant gender differences in daily calcium excretion, which is consistent with literature data (Sargent *et al*., 1993), although there was an increase in daily calcium excretion with age, especially in males ($r = 0.33$, $p = 0.02$).

We have also found that the daily urinary secretion of phosphorus increases as children grow older, especially boys. These data are consistent with the data of Taranta-Janusz (Taranta-Janusz *et al*., 2017) based on the analysis of 3913 healthy children and adolescents aged 2 to 18 years, who revealed that in boys, the absolute daily excretion of phosphorus in the urine was significantly higher, regardless of the way of its expression ($p < 0.001$). The average level of phosphorus in urine increased with age ($p < 0.001$). At the same time, the authors noted that the growing up of children is accompanied by a decrease in the amount of excreted phosphates in terms of body weight and body surface area in both boys and girls. Thalassinos (Thalassinos *et al*., 1970) suggested that the relationship between phosphate excretion and age may be related to changes in dietary habits, as children ate less dairy products and therefore less phosphorus as they got older. Therefore, it is possible that the risk of calcium-phosphorus calculi formation increases with age, especially in males, which requires additional research.

In this research, we did not analyze the daily excretion of metabolites in accordance with the nosological diagnosis, but we plan to do this in the future. However, we believe that the age and gender characteristics of the daily excretion of oxalates, calcium, and phosphates identified by us may reflect the stages of metabolic restructuring of the body in the process of growing up as a whole. In the future, population studies and a possible revision of the existing standards for the excretion of lithogenic substances in urine are needed. Dursun (Dursun *et al*., 2016) suggested that each country should have its own normal reference values for determining the major metabolic risk factor for nephrolithiasis, since regional differences in dietary protein and other nutrient intake may affect normal urinary excretion of oxalate. These researches are necessary not only to predict the risk of developing urolithiasis, but also to improve the diagnosis of conditions such as oxalate, phosphate and other types of nephropathy. Numerous publications report such manifestations of hypercalciuria, urinary tract infection, urgency, abdominal pain, daytime urinary incontinence, or enuresis (Esteghamati *et al*., 2017; Penido *et al*., 2001; Valavi *et al*., 2011).

The disadvantages of our study include the lack of taking into account the body mass index and assessing the nutritional structure, which could have an effect on urine saturation and pH. We also did not pay attention to the supply of vitamin D, which can significantly affect the daily excretion of phosphorus and calcium.

Conclusions

Boys aged 10 years and older have a statistically significantly higher daily urinary excretion of oxalate and phosphorus than girls. This may indicate the gender dependence of some lithogenic risk factors and the need for increased attention to the prevention of nephrolithiasis from early puberty.

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