Original Article

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Predisposing Factors for Nephrolithiasis and Nephrocalcinosis in Cystic Fibrosis

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Abstract

Objective: Cystic fibrosis (CF) is characterized by chronic pulmonary disease, insufficient pancreatic and digestive function, and abnormal sweat concentration. There is controversy about predisposing factors of nephrolithiasis and nephrocalcinosis in patients with cystic fibrosis. We assessed the results of metabolic evaluation in patients with cystic fibrosis and its correlation with nephrocalcinosis.

Methods: Forty five CF patients, mean age 47.1 months, were enrolled in the study. No one had past history of nephrolithiasis and/or nephrocalcinosis. The records were reviewed for clinical characteristics and all patients underwent metabolic evaluation including serum electrolyte measurements and spot urine analysis. Ultrasonography was performed in all patients to detect nephrocalcinosis and urolithiasis.

Findings: Nephrocalcinosis was found in 5 (11%) patients. No patient had clinical symptoms of nephrolithiasis and/or micro/macroscopic hematuria. Metabolic evaluation of the CF patients versus normal reference values showed decreased serum uric acid in 48.8%, elevated serum phosphate in 24.4%, and urine oxalate excretion in 51%. Metabolic evaluation of the nephrocalcinosis positive patients versus nephrocalcinosis negative group showed no statistical difference in serum electrolytes. The mean value of urine calcium excretion was lower in patients with nephrocalcinosis (P=0.001). Despite lack of any significant correlation, higher numerical hyperoxaluria was observed in patients with severe steatorrhea. There was no statistical correlation between steatorrhea and urine calcium as well as oxalate excretion.

Conclusion: Hypocalciuria in the nephrocalcinotic CF patients may be seen. It can be hypothesized that hypocalciuria may be due to a primary defect in renal calcium metabolism in CF patients.

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Key Words: Cystic Fibrosis; Steatorrhea; Nephrocalcinosis; Calcium Oxalate; Urinalysis

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Introduction

Urinary calculi are rare in children but some studies have shown that its incidence is 1% annually in asymptomatic primary school children[1-4]. Metabolic as well as genitourinary anomalies are predisposing factors to urolithiasis[4,5].

Known metabolic risk factors of calcium calculi are hypercalciuria, hyperoxaluria, and increased urinary excretion of uric acid. In addition, decreased excretion of citrate is another important risk factor[6-9]. The main causes of nephrocalcinosis and nephrolithiasis are idiopathic, but it emerges due to certain enzyme deficiency, familial forms and in some known diseases such as cystic fibrosis[9,10].

Cystic Fibrosis (CF) is the most common fatal hereditary illness among the Caucasian population[11]. It is an autosomal recessive, multisystem disorder caused by a mutation of the gene which codes for a protein referred to as the CF transmembrane conductance regulator (CFTR). This protein is located on 7q31[12]. This mutation results in sodium and chloride ion imbalance that causes abnormal thick mucus secretions[12]. Advances in the treatment and management of respiratory and pancreatic disorders have dramatically increased the life expectancy of these patients[13].

Traditionally, it has been accepted that the kidneys were not affected by CF and up until now there has been no demonstrable change in renal function among CF patients. However, the CFTR protein expresses in the renal epithelium and is detected in the proximal tubule, Henle's loop, distal tubule and collecting ducts[12]. Although urolithiasis is usually observed in CF patients over the age of 20, it is not unusual to find it in children [12]. Despite a wide agreement on the association between kidney stones and CF disease, some doubts still exist about the mechanisms of this association[11].

The purpose of the present study was to determine the prevalence of nephrolithiasis or nephrocalcinosis in children with cystic fibrosis and assessment of the importance of risk factors for nephrolithiasis.

Subjects and Methods

In a cross sectional study, 60 CF patients managed in CF outpatient clinic of Dr. Sheikh hospital of Mashhad University of Medical Sciences were included. Patients who had received diuretics and corticosteroids were excluded.

Finally, forty five CF patients consisting of 26 males (aged 6-132 months) and 19 females (aged 9-168 months) were enrolled in the study.

All patients received normal diet as well as vitamins, antibiotics, and mucolytics.

Pancreatic enzymes (Creon) were also given in patients with pancreatic insufficiency. Routine pancreatic enzymes dose was 2000 to 3000 IU/Kg per day. All patients received antibiotics, so bacteriological analysis of stool for *Oxalobacter formigenes* or other oxalate degrading bacteria was not carried out.

Urinary system ultrasound was done for detection of nephrocalcinosis as well as nephro-urolithiasis. Metabolic evaluation was performed to assess stone risk profile.

All cases were evaluated for clinical characteristics and metabolic parameters, including serum electrolytes, blood chemistry and urinalysis along with spot urine analysis, simultaneously. Serum electrolytes consisting of sodium, potassium, chloride, calcium, phosphorus, magnesium as well as blood chemistry including BUN, creatinine, alkaline phosphates and VBG were analyzed. Urine sodium, potassium, chloride, creatinine, magnesium, citrate, oxalate, and calcium were measured by using spot urine examination. 24 hour urine analysis was done only in patients with nephrocalcinosis.

Serum metabolites were determined by using HITACHI 704 Automatic Analyzer and VBG was performed using AVL 993 Blood GAS Analyzer and Nova Biomedical. Urine metabolites such as sodium and potassium were determined using a flame photometer (Seac 20, Italy). Other parameters (Ca, Mg, uric acid, creatinine, citric acid and oxalic acid were assessed by colorimetric methods using auto analyzer (Prestige 24I, Tokyo). Nitroprusside test was done for qualitative evaluation of dibasic amino acids such as cystine in spot urine specimen. The severity of steatorrhea was determined by qualitative analysis of stool fat by Sudan staining.

Steatorrhea was considered as severe when fat droplets were more than 100/hpf in stool smear. The abnormal range of serum metabolites was used as in Özçelik study[14]. Results were compared with age related values. Normal ranges of different urine parameters were extracted[4,15,16](Table 1).

Statistical analysis was performed by using t test, Mann Whitney and Kruskal-Wallis tests. We have compared the mean values after normalization with creatinine (t test). Then we have adjusted the data according to normal values for age (Mann Whitney test).

Informed consent was obtained from parents of all patients. Local Ethics Committee of Islamic Azad University approved this proposal.

Findings

The incidence of nephrocalcinosis, by ultrasonography in our study was 11 % (5 out of 45). No patient had symptoms or signs of nephrolithiasis and microscopic hematuria.

Serum metabolites:

Metabolic evaluation of the cystic fibrosis patients versus normal reference values showed decreased serum uric acid in 48.8% and elevated phosphate level in 24.4% of patients (Table 2).

Plasma creatinine level in 57.8% of CF patients was lower than normal population reference values. Metabolic evaluation of the nephro-calcinosis positive versus negative groups showed no statistical difference in serum electrolytes (Table 3). Other parameters are shown in Table 2.

Urinary metabolic parameters:

Metabolic evaluation of the cystic fibrosis patients versus normal reference values showed elevated urinary oxalate excretion in 51% of patients. Other parameters are shown in Table 2.

We have compared the mean values after normalization with creatinine. No difference was found between groups with respect to sodium, potassium, calcium, magnesium, oxalate and citrate excretion (Table 4: *P*-value of t- test).

Then we have adjusted the data according to normal age-values. This adjustment did not change the results significantly (Table 4: *P*-value of Mann-Whitney test) except for calcium excretion. Mean value of calcium excretion was lower in patients with nephrocalcinosis (*P*=0.001)

Miscellaneous parameters:

Risk factors for hyperoxaluria such as steatorrhea, calcium use and Creon use were evaluated. Severe steatorrhea was found in 40% of patients with nephrocalcinosis. Patients with hyperoxaluria had severe steatorrhea (58.3%).

The correlation of hyperoxaluria with intensity of steatorrhea was not statistically significant (*P*=0.1). There was no statistical correlation between steatorrhea and urine calcium (*P*=0.2) as well as oxalate excretion (*P*=0.1).

Table 1: Definitions of abnormal urine parameters

Abnormal parameters of random urine	Definition
Hypercalciuria	
0 to 6 months	Ca/Cr >0.8 mg/dl
7 to 12 months	Ca/Cr >0.6 mg/dl
>1 yr	Ca/Cr >0.2 mg/dl
Hyperuricosuria	
Infant	Uric acid >3.3 mg/dl GFR
>3 yrs	Uric acid >0.53 mg/dl GFR
Hyperoxaluria	
<1 yr	Oxalate/Cr >0.26 mmol/mmol
1 to 5 yrs	Oxalate/Cr >0.12 mmol/mmol
Nitroprusside	Positive

Table2: Different plasma and urine metabolites in CF patients

	Nephrocalcinotics		All	
Plasma Metabolite	**High**	**Low**	**High**	**Low**
Magnesium (Mg)	0	0	2.2%	6.7%
Alkalin Phosphatase	0	0	4.4%	0
Blood Urea Nitrogen	0	0	2.2%	4.4%
Uric Acid	0	20%	0	48.8%
Sodium (Na)	0	20%	0	40%
Potassium (K)	0	20%	8.9%	4.4%
Chloride (Cl)	0	20%	2.2%	11.1%
Calcium (Ca)	20%	0	4.4%	2.2%
Creatinine (Cr)	0	40%	0	57.8%
Phosphor (P)	40%	0	24.4%	13.6%
pH	20%	20%	6.7%	8.9%
HCO_3	40%	0	17.8%	26.7%
Urine Metabolite				
Na/Cr	0	0	14%	16.3%
Citrate/Cr	0	0	0	2.3%
Nitropruside	0	0	16.3%	0
K/Cr	0	20%	0	25.6%
Mg/Cr	0	20%	4.7%	9.3%
Oxalate/Cr	20%	20%	51.2%	11.6%
Ca/Cr	0	40%	2.3%	4.7%

Discussion

Many studies report higher prevalence of urolithasis in CF adult patients which is 3.5-5.7%[10] compared to 1% in the general population[1-3,12]. We found no renal stones in our pediatric patients.

Katz et al observed microscopic nephro-calcinosis in 35 of 38 specimens (92 percent) from autopsied CF patients; notably, nephrocalcinosis was detected near the time of birth, which supports the hypothesis of the genomic defect. The finding suggests a primary abnormality of calcium metabolism in the kidney[17,18,19]. In Ozcelin et al study the prevalence of nephrocalcinosis by ultrasonography was 23.2%[14]. In our study, nephrocalcinosis was found in 11% of patients.

Oxalate is 10 to 15 times more potent than calcium in increasing the urinary calcium oxalate saturation[20]. Absorptive hyperoxaluria is one of the main causes of nephrocalcinosis in CF patients[21,22]. In our study increased urine oxalate was evident in 51% of patients. We detected that patients with hyperoxaluria had severe steatorrhea. Regarding hyperoxaluria in patients with renal stone, despite lack of any significant correlation, higher numerical hyperoxaluria in patients with stone shows a potential effect on stone formation and needs more evaluation in large population to confirm its correlation.

No statistical difference was observed in nephrocalcinotic versus other patients, indicating a potentiating factor in addition to the increased availability of oxalate in the urine. In a study by Bernd Hoppe et al, the number of patients with hyperoxaluria increased when enzyme substitution decreased. However, hyperoxaluria was also found in the youngest patients receiving the highest enzyme substitution. Patients with or without hyperoxaluria received a comparable

Table 3: Comparing the mean (SD) values for serum parameters in cystic fibrosis patients with and without nephrocalcinosis

Plasma parameters	With nephrocalcinosis	Without nephrocalcinosis	*P*-value (t-test)	*P*-value(Mann Whitney)
Uric acid	4.51±0.8	3.6±1.1	0.1	0.2
Sodium (Na)	138±4.8	138±2.5	0.7	0.3
Potassium (K)	3.98±.7	4.24±0.4	0.5	0.1
Chloride (Cl)	101 ± 9.3	97 ±15.3	0.6	0.5
Creatinine	0.58±.08	.56 ±0.1	0.8	0.4
Magnesium (Mg)	2.54±.2	3.3 ±0.3	0.3	0.7
Calcium (Ca)	10.1±.81	9.9 ±0.6	0.6	0.1
Phosphor (P)	5.22 ±.5	5.1 ±0.8	0.8	0.3
Alkalin Phosphatase	676 ±216	620 ±219	0.6	0.6
Blood Urea Nitrogen	10.8 ±2.3	10.1 ±3.8	0.7	0.8
pH	7.41 ±.09	7.38 ±0.04	0.5	0.9
HCO_3	24.1 ±3.8	21.5 ±3.3	0.1	0.08

enzyme dosage, and all had normal fecal fat excretions, hence the amount of substituted enzyme seemed not to be of great importance as a cause of hyperoxaluria[20]. In our study there was no relation between Creon dosage and urinary oxalate level (*P*=0.5).

Absence of *Oxalobacter formigenes* may contribute to hyperoxaluria[23]. Sidhu et al reported absent *Oxalobacter formigenes* in the stool of cystic fibrosis patients. They concluded that absence of this normal organism of the enteric flora leads to increased absorption of oxalate and prolonged widespread use of antibiotics in cystic fibrosis population may induce permanent decolonization[24]. Assessment of Oxalobacter colonization cannot be possible because of using high expansion antibiotics[11].

Some articles have shown that hypercalciuria in CF patients is a renal stone risk factor[25,26]. Also it was reported that hypocalciuria can protect cystic fibrosis patients against nephrolithiasis[22,23]. Bohles and Michalk suggested that calcium supplementation may increase the risk of stone formation[27].

However, in cystic fibrosis according to the fat malabsorption, bile salt and fatty acids fail to be reabsorbed in intestinal tract, so calcium binds to them through saponification. It can decrease intestinal calcium absorption and therefore may lead to high levels of oxalate absorption which

Table 4: Comparing the mean (SD) values for urine parameters in cystic fibrosis patients with and without nephrocalcinosis

Plasma parameters	With nephrocalcinosis	Without nephrocalcinosis	*P.* value (t-test)	*P.* value (Mann Whitney)
Sodium/ Creatinine	13.86±4.6	34.2±42.9	0.3	0.9
Potassium/ Creatinine	15.8 ±17.8	15.1 ±14.0	0.9	0.8
Magnesium / Creatinine	0.48 ±.3	0.76 ±0.4	0.2	0.3
Oxalate/ Creatinine	0.12 ±.17	0 .21 ±0.3	0.6	0.1
Calcium/ Creatinine	0.25 ±.2	0 .33 ±0.2	0.5	0.001
Citrate/ Creatinine	6.98 ±8.1	5.72 ±4.5	0.6	0.7

produces hypocalciuria as well as hyperoxaluria[28,29].

In our study serum calcium was within normal limits in each group. Importantly urine calcium was decreased in all groups but it was statistically significantly lower in nephrocalcinotics. There was no significant relation between urine calcium, hyperoxaluria and steatorrhea. Also there was no significant relation between hyperoxaluria and steatorrhea. This maybe due to data limitation.

It was noted that severe pyridoxine deficiency decreases alanine-glyoxylate aminotransferase (AGT) activity. This reduced activity may increase oxalate Generation[30,31]. However, severe vitamin B6 deficiency was unlikely in CF patients given multivitamins[11].

Although hyperuricemia may be related to the significant ingestion of pancreatic enzymes, increased catabolism and increased oxidative stress in CF patients[11], in our study the serum uric acid of CF patients with and without nephrocalcinosis was low (20% and 48.8% respectively).

Knowing the way in which kidney stones are produced in CF, it is important to carry out periodical studies on these patients in order to control the possible lithogenic factors previously mentioned, to correct possible intestinal malabsorption and to take dietary measures, such as reducing the ingestion of oxalate, introducing citrate supplements in certain cases and recommending an increased intake of liquids which may prevent the formation of kidney stones[12].

In fact, the most important difficulty for evaluation of urine chemistry in these patients is the absence of a specific reference range. In addition, they are younger and often underweight compared with healthy individuals, and are given high-energy and high-protein diets to prevent malnutrition[21]. We could not perform urinary analysis for urate and cystine.

Also we did not obtain 24 hour urine analysis except for nephrocalcinotics. All of these problems and small number of our cases may be the confounding and limiting factors in the interpretation of the results.

Conclusion

We can hypothesize that hypocalciuria may be due to a primary defect in renal calcium metabolism in CF patients, although it is difficult to rule out all of the secondary causes of nephrocalcinosis in this study. For evaluation of this hypothesis, further controlled studies are needed.

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Conflict of Interest: None

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