Hypercalciuria and nephrocalcinosis in cystic fibrosis patients

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The objective of this study was to determine the frequency of nephrocalcinosis and hypercalciuria in cystic fibrosis (CF) patients, and to search possible causes of this phenomenon. Forty-three CF children (24 boys, 19 girls; mean age 64.9 months, range 5 months-18 years) were included in this study. Plasma sodium, potassium, chloride, BUN, creatinine, calcium, phosphorus, magnesium, alkaline phosphatase; spot urine sodium, potassium, chloride, creatinine, calcium, magnesium; and serum 25-hydroxyvitamin-D levels were measured in all patients. Urine samples were examined for microscopic hematuria. Fractional sodium, potassium, chloride excretion and estimated glomerular filtration rate (GFR) were calculated. All patients underwent renal ultrasonography.

Hypercalciuria, nephrocalcinosis and microscopic hematuria were detected in 15 patients (34.2%), 10 patients (23.2%) and two patients (5%), respectively. There was no significant but borderline correlation between 25-hydroxyvitamin-D levels and hypercalciuria (r: 0.308, p:0.05). There were no correlations between Shwachman clinical scoring system results and hypercalciuria (r: 0.221, p: 0.148) and age and hypercalciuria (r: -0.229, p: 0.135). Patients with chronic Pseudomonas colonization showed no hypercalciuria or nephrocalcinosis. There was no difference for plasma biochemical results, renal function tests, hypercalciuria and nephrocalcinosis between CF patients who had or had not experienced pseudo Bartter's syndrome (PBS) before. There was no relation between detected CF mutations of the patients and hypercalciuria and nephrocalcinosis. These results suggested that it is a primary abnormality of calcium metabolism in the kidney.

Key words: cystic fibrosis, hypercalciuria, nephrocalcinosis, pseudo Bartter's syndrome.

Cystic fibrosis (CF) is an inherited disorder causing pancreatic, pulmonary and sinus disease in patients. It is caused by defects in the CF transmembrane conductance regulator (CFTR) gene, which encodes a protein that functions as a chloride channel and is regulated by "cyclic adenosine monophosphate" (camp) dependent protein kinase[1]. Considering the special role of the kidney for fluid and ion homeostasis, alteration of calcium metabolism would be expected in a CF kidney. There are some controversial studies about nephrocalcinosis and hypercalciuria in CF patients. Katz et al.[2] found a high frequency of nephrocalcinosis and hypercalciuria in CF patients, and they postulated that "this is a primary renal leak of calcium". On the other hand, in some other studies, hypercalciuria/nephrocalcinosis frequency was found no higher than other chronic debilitating diseases[3,4].

The objective of the present study was to determine the frequency of nephrocalcinosis and hypercalciuria in CF children, and to search for possible causes of this phenomenon.

Material and Methods

Forty-three consecutive (CF) children (24 boys, 19 girls), who came for control visits to the out patient clinic of Hacettepe University, İhsan Doğramacı Children's Hospital, Pediatric Pulmonology Division during the study period,

were included in this study. CF was diagnosed by at least two sweat chloride values higher than 60 mEq/L, along with the presence of physical and laboratory findings compatible with the disease. Mean age was 64.9 months (range 5-216 months). Mean Shwachman clinical scoring system result was 78.16 (range 40-98). Chronic Pseudomonas colonization was noted in five patients. All the patients received 3 mEq/kg extra sodium chloride and 800 IU/day vitamin D orally. Dietary calcium content was within normal range (426-1137 mg) for all patients. None of the patients received steroids or diuretics before, or aminoglycoside antibiotics during, the study period.

Pseudo Bartter syndrome (PBS) was diagnosed in the CF patients when they showed hypoelectrolytemia with alkalosis, and elevated renin and aldosterone levels in serum. In 16 patients at least one PBS attack had been diagnosed before this study. During the study period none of the patients showed PBS and mean period from their last PBS attack was 18 months (2-76 months).

Plasma sodium (138-145 mEq/L), potassium (3.4-4.7 mEq/L), chloride (95-110 mEq/L), BUN (5-18 mg/dl), creatinine (0.6-1.2 mg/dl), uric acid (3.4-7 mg/dl), calcium (8.8-10.8 mg/dl), phosphorus (4.5-5.5 mg/dl), magnesium (1.8-3 mg/dl), and alkaline phosphatase (250-1000 U/L); and spot urine sodium, potassium, chloride, creatinine, calcium, and magnesium measurements were performed in all patients by autoanalyzer (Hitachi 911 automatic analyzer). Serum 25-hydroxyvitamin-D levels were measured by RIA kit (Incstar-USA) in all patients (N: 10-40 ng/ml). Urine samples were examined for microscopic hematuria. Five or more erythrocytes in one microscopic area was accepted as hematuria. Fractional sodium, potassium, and chloride excretion were calculated using plasma and spot urine sodium, potassium, and creatinine levels. Twenty-four hour urine collection could not be performed because of the patients' age group. Therefore estimated glomerular filtration rate (GFR) was measured using plasma creatinine as described in the literature[5].

Hypercalciuria was defined as urine calcium/creatinine levels above 0.25 mg/mg creatinine, and hypermagnesuria was magnesium/creatinine levels above 0.27 mg/mg creatinine[6].

The two most common mutations of the CFTR gene in our population, delta F 508 and 1677 delTA, were tested in all 43 patients[7]. Some but not all of the untyped alleles could be sequenced to search for unidentified mutations. Patients were divided into three groups according to the genotype, as group 1: homozygous for the delta F508 mutation (n:8); group 2: compound heterozygous for delta F508 mutation (n: 15, 2 patients-dF508/1677delTA; 2 patients-dF508/2789+5G-A; each dF508/W1282X, dF508/N1303K, dF508/R1066L, dF508/4374+1 G-A, dF508/148T; 6 patients-dF508/unknown) and, group 3: other known mutations (n: 20; A96E/A96E, R347/R347H, M152V/M152V, 2183+5G-A/D1152H, W496/2181 delA, 2183A-G, G85E/R334W, 1677delTA/E92K, 621+1G-T/621+1G-T, N1303K/W1098XG-A, 1677delTA/unknown, R1162X/unknown, G85E/unknown, 8 unknown/unknown).

All patients underwent renal ultrasonography. A scoring system was used for evaluationg nephrocalcinosis sonographically. Cortical hyperechogenicity was scored as type 1, medullary hyperechogenicity as type 2, and cortical and medullary hyperechogenicity as type 3.

Informed consent was obtained from parents of all participating children.

Data are given as mean±SEM. Statistical analyses were performed by using SPSS computerized statistics program with Mann Whitney-U test and correlations with Pearson correlation test. At two-tailed p value of less than 0.05 was considered significant.

Results

Biochemical data of the patients are presented in Tables I and II. Hypercalciuria, nephrocalcinosis and microscopic hematuria were detected in 15 patients (34.2%), 10 patients (23.2%), and two patients (5%), respectively. All patients with nephrocalcinosis showed type 2 (medullary) hyperechogenicity. All 10 patients with nephrocalcinosis and two patients with hematuria also had hypercalciuria.

There was no significant, but borderline, correlation between 25-hydroxyvitamin-D levels and hypercalciuria (r: 0.308, p: 0.05) (Fig. 1). There was no correlation between Shwachman clinical scoring system results and hypercalciuria (r: 0.221; p: 0.148). Also, no correlation was

detected for age of the patients and hypercalciuria (r: -0.229; p: 0.135). Patients with chronic Pseudomonas colonization did not show hypercalciuria or nephrocalcinosis. There was no difference in plasma biochemical results, renal function tests, hypercalciuria and nephrocalcinosis between CF patients with and without PBS (Tables III and IV). No significant difference was observed for any mutation group in the CF patients with or without hypercalciuria (p: 0.194) or nephrocalcinosis (p: 0.242).

Table I. Biochemical Data in Serum of the Patients

	mean (±SD)
pH	7.35 (±0.04)
HCO_3 (mEq/L)	20.87 (±2)
Na (mEq/L)	143.53 (±3.52)
K (mEq/L)	4.63 (±0.48)
CL (mEq/L)	105.84 (±3.39)
Osmolarity (mOsm/L)	299.50 (±17.09)
BUN (mg/dl)	12.02 (±5.11)
Cre (mg/dl)	0.56 (±0.14)
Uric acid (mg/dl)	4.15 (±1.40)
Ca (mg/dl)	9.89 (±0.63)
P (mg/dl)	5.32 (±0.77)
Alkaline phosphatase (U/L)	289.58 (+98.65)
25-hydroxyvitamin-D (ng/ml)	16.93 (±7.52)

HCO_3, bicarbonate; Na, sodium; K, potassium; CL, chloride; BUN, blood urea nitrogen; Cre, creatinine; Ca, calcium; P, phosphorus.

Table II. Biochemical Data in Urine of the Patients

	mean (±SD)
pH	5.63 (±1.04)
Density	1019.23 (±6.52)
Osmolarity (mOsm/L	671.22 (±281.63)
Ca/Cr (mg/mg)	0.17 (±0.10)
Mg/Cr (mg/mg)	0.13 (±0.12)
FeNa (%)	0.78 (±0.80)
FeK (%)	18.80 (±13.51)
FeCI (%)	1.02 (±0.80)
EGFR (ml/min 1.73 m^2)	101.46 (±25.33)

Ca/Cr, calcium/creatinine; Mg/Cr, magnesium/creatinine; FeNa, fractional sodium excretion; FeK, fractional potassium excretion; FeCI, fractional chloride excretion; EGFR, estimated glomerular filtration rate.

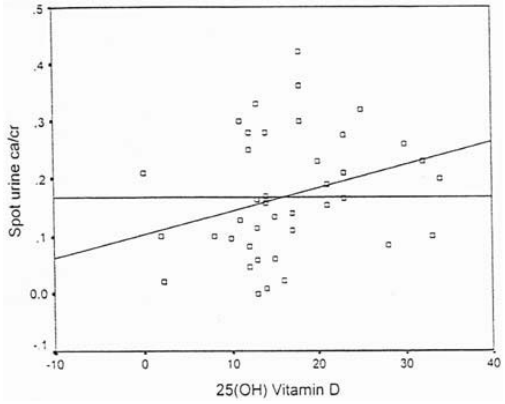

Fig. 1. Relation between spot urine calcium/creatinine levels and 25-hydroxyvitamin-D levels (r: 0.308, p: 0.05).

Table III. Comparative Biochemical Data in Serum of the Patients in Two Groups

	Group I	Group II	p
pH	7.34±0.47	7.35±0.036	0.48
HCO_3 (mEq/L)	20.18±2.38	21.28±1.64	0.09
Na (mEq/L)	143.37±3.55	143.62±3.23	0.92
K (mEq/L)	4.60±0.44	4.64±0.512	0.50
CL (mEq/L)	105.68±2.54	105.9±23.82	0.79
Osmolarity (mOsm/L)	294.64±4.06	277.37±87.22	0.13
BUN (mg/dl)	14.12±6.37	0.77±3.82	0.10
Cre (mg/dl)	0.49±0.085	0.59±0.158	0.06
Uric acid (mg/dl)	4.26±1.46	4.08±1.38	0.76
Ca (mg/dl)	9.83±0.819	9.91±0.5	0.87
P (mg/dl)	5.43±0.86	5.25±0.72	0.63
Alkaline phosphatase (U/L)	283.73±95	252.38±142	0.30
25-hydroxyvitamin-D (ng/mL)	19.25±8.29	14.67±7.2	0.16

Group I: CF patients who experienced Pseudo-Bartter's syndrome (PBS) before.
Group II: CF patients who did not experience Pseudo Bartter's syndrome (PBS) before.

HCO_3, bicarbonate; Na, sodium; K, potassium; CL, chloride; BUN, blood urea nitrogen; Cre, creatinine; Ca, calcium; P, phosphorus, $p<0.05$ is significant.

Table IV. Comparative Biochemical Data in Serum of the Patients in Two Groups

	Group I	Group II	p
pH	5.78±1.27	5.33±1.37	0.57
Density	1020.62±5.43	1018.40±7.04	0.36
Osmolarity (mOsm/L)	699.64±303.35	601.60±318	0.36
Ca/Cr (mg/mg)	0.20±0.1	0.15±0.10	0.16
Mg/Cr (mg/mg)	0.13±0.15	0.11±0.20	0.86
FeNa (%)	0.57±0.65	0.90±0.86	0.08
FeK (%)	21.07±17.94	17.44±10.19	0.95
FeCI (%)	0.82±0.68	1.14±0.85	0.14
EGFR (ml/min/1.73 m^2)	94.92±19.54	105.32±27.8	0.19

Group I: CF patients who experienced Pseudo-Bartter's syndrome before.
Group II: CF patients who did not experience Pseudo-Bartter's syndrome before.

Ca/Cr, calcium/creatinine; Mg/Cr, magnesium/creatinine; FeNa, fractional sodium excretion; FeK, fractional potassium excretion; FeCI, fractional chloride excretion, EGFR, estimated glomerular filtration rate; $p<0.05$ is significant.

Discussion

Several recent studies have been undertaken in order to evaluate whether renal calcium metabolism is altered in patients with CF. In our study we detected high frequency of hypercalciuria (34.2%) and nephrocalcinosis (23.2%) in CF patients. In 1988 Katz et al.[2] demonstrated microscopic nephrocalcinosis in 35 of 38 renal specimens obtained from autopsy of CF patients, including a baby stillborn near the time of birth, and in none of the 10 autopsy controls, all of whom died as a result of acute trauma. Also, hypercalciuria was detected in five of the 14 CF patients. On this basis they postulated that there is primary abnormality of calcium metabolism in the kidney of CF patients. On the other hand Gruskin et al.[3] found no differences in calcium excretion between CF patients and normal controls. Bentur et al.[4] detected normal urinary calcium excretion in 30 of the 34 CF patients; none of the 17 patients examined by renal ultrasonography had nephrocalcinosis. They also performed a similar study on patients who died as a result of chronic debilitating disease. Focal deposits of calcium were seen in five of the 14 CF patients, and in six of 15 control patients. They concluded that microscopic nephrocalcinosis and hypercalciuria in CF patients have, most likely, secondary epiphenomenon related to preterminal events.

It is known that many factors can alter calcium excretion in CF patients. In our study, all of the patients' glomerular and tubular functions, and plasma calcium, phosphorus and alkaline phosphatase levels were within normal limits. None of the patients used drugs that altered calcium metabolism. Dietary calcium levels were within normal limits. Another contributing factor to development of hypercalciuria and nephrocalcinosis could be vitamin D supplementation. However 25-hydroxyvitamin-D levels in our patients were detected as normal or below normal in spite of the fact that they were receiving 800 IU/day vitamin D.

One of the possible causes for the abnormal calcium excretion is high sodium intake. However, this was not so in our patients. All the patients had received 3 mEq/kg extra sodium chloride in their diet, but their serum sodium and fractional sodium excretion levels were within normal limits.

Immobilization is usually mentioned among other causes of hypercalciuria. Our patients' Shwachman clinical scores were generally high, therefore, none was immobile because of the disease, and there was no relation between clinical score results and hypercalciuria.

Patients with CF usually have essential fatty acid (EFA) deficiency. It is a well known fact that EFA deficiency in rats causes functional and morphological changes in the kidney, such as decreased urinary volume, hematuria, and nephrocalcinosis[8]. EFA measurement could not be performed on our patients.

Hypercalciuria and nephrocalcinosis have also been described in patients with PBS. Hypochloremic metabolic alkalosis with hyponatremia, hypokalemia, hypochloremia, and elevated plasma renin and aldosterone levels are the characteristic biochemical picture of Bartter's syndrome[9]. There are some other

disorders including CF, in which the metabolic findings may mimic Bartter's syndrome, called PBS[10-14]. Loss of electrolytes through sweating plays a primary role in the appearance of PBS in patients with CF[15,16]. Vomiting, diarrhea, and feeding with inadequate salt diet also contribute to development of PBS[17,18]. PBS was not uncommon in our CF patients[19]. Rodriguez-Soriano et al.[20] reported 30 patients who showed PBS because of chloride deficiency. They showed a significant elevation in the serum concentrations of calcium and phosphate with high urinary excretions of calcium and magnesium continuing, even after almost-complete recovery of the remaining biochemical disturbances due to chloride deficiency. The authors also pointed out the potential risk of developing nephrocalcinosis after dietary chloride deficiency. Nephrocalcinosis was reported in six fatal cases of chloride-depletion alkalosis related to pyloric stenosis[21]. To date, no findings have been described regarding long-term renal function and nephrocalcinosis in CF patients who experienced PBS. To find the effect of PBS on hypercalciuria and nephrocalcinosis in CF patients, we compared renal functions, plasma levels of calcium and phosphorus, hypercalciuria and nephrocalcinosis in CF patients who experienced at least one known PBS attack in their past history, with those who did not. We could not find any statistical difference, regarding hypercalciuria and nephrocalcinosis, between the two groups.

Elevated excretion of other lithogenic substances, e.g. oxalate, glycolate and uric acid, has been reported in CF patients before[22,23]. These measurements could not be performed in our patients.

Although the number of our patients was too small to reach a definitive conclusion, we did not detect any relation between a specific CF genotype and the appearance of hypercalciuria and nephrocalcinosis in our patients. This suggests that other genetic or environmental factors may influence the development of hypercalciuria or nephrocalcinosis. Recently, some studies on hypercalciuria and nephrolithiasis disorders showed the relation between these disorders and chloride channels (CLC)[24,25]. These disorders of hypercalciuric nephrolithiasis (Dent's disease, X-linked recessive nephrolithiasis, X-linked recessive hypophosphatemic rickets, idiopathic low molecular weight proteinuria associated with hypercalciuric nephrocalcinosis in Japanese children) have been established as sharing a common genetic etiology by demonstrating mutations in the renal chloride channel gene (CLCN5). It is not known, but there are some hypotheses regarding how mutations in the renal chloride channel result in low-molecular weight proteinuria and hypercalciuria. CFTR is different from other CLC, functioning as a cAMP-activating chloride channel of low conductance, but there is some evidence about the control role of CFTR on the regulation of other chloride channels[26]. With these findings we can suggest that the high incidence of hypercalciuria and nephrocalcinosis in CF patients may be the result of disregulation of CFTR expressed in kidney tubules or the effect of CFTR on the other chloride channels.

In conclusion, we detected a high frequency of hypercalciuria (34.2%) and nephrocalcinosis (23.2%) in our CF patients. There was no detected relation between these findings and possible causes of hypercalciuria and nephrocalcinosis. The results suggest that it is a primary abnormality of calcium metabolism in the kidney.

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