



AN INFANT WITH IDIOPATHIC HYPERCALCIURIA AND NEPHROLITHIASIS ASSOCIATED WITH CYP24A1 ENZYME POLYMORPHISM: A CASE REPORT

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SUMMARY – CYP24A1 is an enzyme that inactivates vitamin D and encodes vitamin D 24-hydroxylase. Mutations in this enzyme have been linked with idiopathic infantile hypercalcemia, nephrolithiasis, and nephrocalcinosis. Genetic testing for this mutation should be considered in the presence of calciuria, elevated serum calcium, elevated 1,25-dihydroxyvitamin D, and suppressed parathyroid hormone. We present a previously healthy eight-month-old male infant with macrohematuria, hypercalciuria (6 mg/kg/24 h), albuminuria (54 mg/24 h) and left-sided nephrolithiasis found on urinary tract ultrasound. The values of alpha 1 microglobulin, parathyroid hormone, vitamin D, serum electrolytes, amino acids, glycols, oxalates and citrates in urine, as well as coagulation tests were normal. Genetic testing excluded suspected Dent's disease but confirmed heterozygous missense variant CYP24A1 c.469C>T, p.(Arg157Trp) classified as polymorphism. He was treated with hydrochlorothiazide and potassium citrate. Children presenting with hypercalcemia, hypercalciuria and nephrolithiasis should be tested because of the importance of recognition, genetic diagnosis and proper treatment of CYP24A1 mutations that can present with a wide range of phenotypic presentations, from asymptomatic to chronic renal disease.

Key words: *Idiopathic hypercalciuria; Nephrolithiasis; Macrohematuria; CYP24A1; Vitamin D*

Introduction

Over the past decade, there has been an increased focus on the benefits of vitamin D for the maintenance of bone health and possible prevention of chronic diseases such as cancer and cardiovascular disease. Two steps of hydroxylation, the first in the liver and the second in the kidneys, are required to produce the active form of vitamin D, which plays an important role in calcium and phosphate metabolism. Vitamin D catabolism follows two different pathways, beginning with 24- or 23-hydroxylation and including several steps of hydroxylation catalyzed by the mitochondrial enzyme, 25-hydroxyvitamin D 24-hydroxylase. CYP24A1 enzyme is expressed in most vitamin D target

tissues, inactivates vitamin D, and encodes function of vitamin D 24-hydroxylase¹. Mutations in CYP24A1 can present with a broad spectrum of clinical manifestations caused by the increased values of vitamin D active form, including idiopathic infantile hypercalcemia, nephrolithiasis, and nephrocalcinosis in children and adults, which can lead to chronic renal insufficiency. Although these mutations are rare, genetic testing should be done in case of hypercalciuria, hypercalcemia, increased values of 1,25-dihydroxyvitamin D, and suppressed parathyroid hormone².

Case Report

A previously healthy eight-month-old male infant was admitted to the Nephrology Department with macroscopic hematuria. He was born full-term by cesarean section because of cephalopelvic disproportion. The pregnancy was burdened by gestational diabetes that was under control with a diet. The infant was un-

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Fig. 1. Ultrasound showing kidney stone in the left kidney, 6 mm in diameter.

der supervision of cardiologist because of the ventricular septal defect. He was breastfed for up to four months, and afterwards fed with milk formula, and some mixed fruits and vegetables. The family history was inconspicuous and negative for nephrolithiasis and any urinary tract abnormalities. On admission to the department, he was in good general condition, afebrile, with normal vital parameters. His body weight was 8.05 kg (15th centile, Z-score: -1.02), length 76 cm (94th centile), and head circumference 46.1 cm (77th centile). Except for the heart murmur III/VI, there were no signs of illness or any other deviations in his clinical examination.

In laboratory findings, there were 90% dysmorphic erythrocytes in the urine and elevated calcium/creatinine ratio (1.5 mmol/mmol, 2.1 mmol/mmol; reference value for age 7-12 months: <1.5 mmol/mmol)³. Urinary tract ultrasound revealed a kidney stone of 6 mm in diameter in the middle cup of the left kidney with some acoustic shadow behind (Figs. 1 and 2). The values of alpha-1-microglobulin, parathyroid hormone, vitamin D, serum electrolytes, antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), amino acids, glycols, oxalates and citrates in the urine and coagulation tests were normal. Immunoglobulin blood test, C3, and C4 levels were normal.



Fig. 2. Ultrasound showing kidney stone in the middle cup of the left kidney with some acoustic shadow behind.

With parents' informed consent, a dwelling catheter was placed to collect 24-hour urine sample, in which we found elevated calcium values of 6 mg/kg/24 h (reference value: <4 mg/kg/24 h or 0.1-0.125 mmol/kg/day) and albuminuria of 54 mg/24 h (reference value: <30 mg/24 h)⁴. Follow-up ultrasound showed the same finding of left-sided nephrolithiasis without any signs of nephrocalcinosis or obstruction. Initially, the patient was treated with hydrochlorothiazide in a dose of 1 mg/kg once a day, with recommendation of increased fluid intake (at least 800 mL) with higher citrate content and a low-salt diet. Genetic testing excluded suspected Dent's disease but confirmed heterozygous missense variant CYP24A1 c.469C>T, p.(Arg-157Trp) classified as a polymorphism. After one month of therapy, the patient was examined at follow-up appointment. There was no macroscopic hematuria, the infant was healthy, control laboratory results were in the normal range, calcium/creatinine ratio was normal (0.3 mmol/mmol), and ultrasound of the urinary tract was unchanged. Given the findings of genetic testing, we omitted vitamin D supplementation. Hydrochlorothiazide therapy was continued, as well as regular outpatient follow-up. Approximately a year later, we lowered the dose of hydrochlorothiazide to 0.5 mg/kg. Two months later, the patient was admitted for regular follow-up and 24-hour urine sample collection. He was one year and nine months old; body weight was 12.5 kg (86th centile) and body height 90 cm (98th centile). Follow-up findings showed hyper-

calciuria of 9 mg/kg/24 h, with normal serum electrolytes, acid-base status, vitamin D and parathyroid hormone values. The calcium/creatinine ratio was 2.5 mmol/mmol (age-specific reference value: <1.5 mmol/mmol). Therapy was changed to potassium citrate in a dose of 2 mmol potassium ion/kg/day divided into four daily doses. We recommended fluid intake with higher citrate content of about 900 mL a day and a low-salt diet. Subsequent clinical follow-up was without any signs of hypercalciuria, with all tests within the reference values, and it was considered a good response to potassium citrate therapy. Ultrasound of the urinary tract remained unchanged. During the follow-up, there was no relapse of macrohematuria, the infant was in good general condition. The child is now 2 and a half years old and we are still following him every 3-4 months.

Discussion

Our patient presented with macroscopic hematuria, underlying idiopathic hypercalciuria, and nephrolithiasis. The prevalence of hypercalciuria in healthy infants has been reported at 3%-7%. However, the incidence of hypercalciuria in patients with urolithiasis is much higher, and it is generally considered the most common identifiable metabolic risk factor for calcium nephrolithiasis⁵. Hypercalciuria is defined as daily urinary excretion of more than 4 mg calcium/kg/day. This definition is somewhat more useful in the pediatric age group if the child is over two years of age. Reference values for hypercalciuria and albuminuria in infants and children between one and two years of age are not defined, most likely due to difficult 24-hour urine sample collection, and the need for dwelling catheter placement. Another clinically useful definition, especially in infant age, is the random or spot urinary calcium/creatinine ratio⁶. Young children and infants tend to have higher urinary calcium excretion and lower urinary creatinine levels, so the suggested normal limits for calcium/creatinine ratios differ by age as follows: up to six months of age, less than 0.8 mg/mg (<2.24 mmol/mmol); six to twelve months of age, less than 0.6 mg/mg (<1.5 mmol/mmol); and 24 months and older, less than 0.2 mg/mg (<0.56 mmol/mmol)^{3,6}. Since our patient had idiopathic hypercalciuria and nephrolithiasis in the infant age, he underwent genetic testing for Dent's disease, X-linked disorder character-

ized by low molecular weight proteinuria, hypercalciuria, nephrolithiasis, and nephrocalcinosis with progressive course to the end-stage chronic renal disease⁷. Tests excluded Dent's disease, but confirmed the infant to be heterozygous for missense variant CYP24A2 c.469C>T, p.(Arg157Trp) of uncertain significance and inherited as an autosomal recessive trait. This variant is described in publications with other variants frequently found in patients presenting with hypercalcemia and nephrocalcinosis. It is classified as a polymorphism, and most of the heterozygous patients are asymptomatic^{1,8}. The clinical significance of CYP24A1 c.469C>T, p.(Arg157Trp) is uncertain, and recent findings emphasize the importance of recognition, genetic diagnosis, and proper treatment of CYP24A1 mutations¹. In patients with hypercalcemia who are heterozygous for CYP24A1 mutations, restriction of vitamin D intake is recommended; in the infant age, vitamin D prophylaxis should be stopped with correction of dietary habits and increased fluid and citrate intake⁷. Vitamin D is given to all infants prophylactically (400 IU), regardless of the climate, living region, infant's diet, or family history. However, the needs differ among individuals, so each child should be evaluated and given individualized prophylactic dose of vitamin D. In children with hypercalciuria and/or nephrolithiasis, vitamin D supplementation should be avoided, and in patients with underlying CYP24A1 mutations, increased vitamin D intake can trigger the onset of symptoms^{5,9,10}.

Initially, our patient was treated with hydrochlorothiazide (1 mg/kg) with good response to therapy, but when we tried to lower the dose, he had significant hypercalciuria and elevated calcium/creatinine ratio again. Recently, a correlation between the use of hydrochlorothiazide and non-melanoma skin cancer has been reported. The results presented show a significant risk of non-melanoma skin cancer when hydrochlorothiazide was used for more than 2 years¹¹. Because of the poor therapy effect of lower dose and the known risk of non-melanoma skin cancer, therapy was changed to potassium citrate (2 mmol potassium ion/kg/day), and so far, we have observed a good therapy effect. There was no hypercalciuria, calcium/creatinine ratio was normal, and all other control tests were in the reference values. We consider it to be a good response to the potassium citrate therapy, and it was unlikely to expect spontaneous resolving of hypercalciuria. Chil-

dren presenting with hypercalcemia, hypercalciuria and nephrolithiasis should be tested because of the importance of recognition, genetic diagnosis and proper treatment of CYP24A1 mutations that can present with a wide range of clinical presentations, from asymptomatic to chronic renal disease.

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Sažetak

IDIOPATSKA HIPERKALCIURIJA I NEFROLITIJAZA U DOJENČETA S DOKAZANIM POLIMORFIZMOM ENZIMA CYP24A1 – PRIKAZ BOLESNIKA

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CYP24A1 je enzim koji inaktivira vitamin D i regulira funkciju 25-OH-D3-24-hidroksilaze, a njegove mutacije uzrokuju idiopatsku infantilnu hiperkalcemiju, nefrolitijazu i nefrokalcinozu. Genetsko testiranje trebalo bi učiniti u slučaju hiperkalcemije, hiperkalcemije, povišenih vrijednosti 1,25 dihidroksivitamina D i sniženih vrijednosti paratireoidnog hormona. Prikazujemo slučaj prethodno zdravog muškog dojenčeta u dobi od 8 mjeseci s makrohaturijom, hiperkalcemijom (6 mg/kg/24 h), albuminurijom (54 mg/24 h) i ultrazvučno vidljivim nefrolitom u lijevom bubregu. Vrijednosti alfa 1 mikroglobulina, paratireoidnog hormona, vitamina D, elektrolita u serumu, aminokiselina, glikola, oksalata i citrata u mokraći te koagulacijskih testova bile su uredne. Učinjeno je genetsko testiranje zbog sumnje na Dentovu bolest koja nije potvrđena. Bolesnik je heterozigotan za *missense* varijantu CYP24A1 c.469C>T, p.(Arg157Trp) te je klasificirana kao polimorfizam. Liječen je hidroklorotiazidom, potom kalij citratom. Kod djece s hiperkalcemijom, hiperkalcemijom i nefrolitijazom treba provesti genetsko testiranje zbog važnosti prepoznavanja, dijagnoze i pravilnog liječenja mutacija CYP24A1 koje su širokog kliničkog spektra, od asimptomatskog do kronične bubrežne insuficijencije.

Ključne riječi: *Idiopatska hiperkalcemija; Nefrolitijaza; Makrohaturija; CYP24A1; Vitamin D*