SERBIAN ARCHIVES OF MEDICINE Est.1872

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Paper Accepted* **ISSN Online 2406-0895**

Case Report / Приказ случаја

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Hypercalciuria caused by *CYP24A1* mutation – fourteen years of the patient's follow-up

Хиперкалциурија изазвана мутацијом *CYP24A1*: четрнаест година праћења

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Received: January 16, 2017
Accepted: February 22, 2017
Online First: March 31, 2017
DOI: 10.2298/SARH170116095P

* **Accepted papers** are articles in press that have gone through due peer review process and have been accepted for publication by the Editorial Board of the *Serbian Archives of Medicine*. They have not yet been copy edited and/or formatted in the publication house style, and the text may be changed before the final publication.
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SUMMARY

Introduction Recently, inactivation mutations of *CYP24A1*, the gene encoding vitamin D 24-hydroxylase, were identified in hypercalciuric nephrolithiasis and nephrocalcinosis.

The aim of this work was to describe the long term follow-up of a patient with hypercalciuric nephrolithiasis caused by *CYP24A1* mutations.

Case Outline A male Montenegro patient first presented with microhematuria at the age of 5 years. Hypercalciuria was documented and for some time he was treated by hydrochlorothiazide. After 12 years the patient presented with macrohematuria and left sided nephrolithiasis. He was found to have intermittent borderline hypercalcaemia, suppressed parathyroid hormone (PTH), hypercalciuria and increased plasma 25-hydroxy vitamin D [25(OH)D_3]. The patient denied any vitamin D supplementation and all other causes of hypercalcemia were ruled out. The positive family history for nephrolithiasis (both parents and grandmother) and the similar biochemical abnormalities detected in father and son, pointed to an inherited disorder. A homozygous mutation in *CYP24A1* (E143del) was found in patient and his father, while mother is heterozygous. During follow-up of two years the patient underwent four extracorporeal shockwave lithotripsies, he was advised to increase water intake, and to avoid sunlight exposure. At the end of follow-up he was asymptomatic, and his renal ultrasound was normal, as well as his renal function, but hypercalciuria and low PTH levels persisted.

Conclusion Hypervitaminosis D should be considered in children with idiopathic hypercalciuria, nephrolithiasis and nephrocalcinosis of unknown etiology. Recognition of *CYP24A1* mutations in these patients may help to decrease the serious consequences by avoiding vitamin D supplements and excessive sun exposure.

Keywords: Hereditary nephrolithiasis, nephrocalcinosis, hypervitaminosis D, idiopathic hypercalcemia

Сажетак

Увод Недавно је, као узрок хиперкалциуричне нефролитијазе и нефрокалцинозе откривена инактивациона мутација *CYP24A1*, гена који кодира витамин Д 24-хидроксилазу.

Циљ овог рада је опишемо дуготрајно праћење болесника са хиперкалциуричном нефролитијазом изазваном *CYP24A1* мутацијом.

Приказ болесника Дечак из Црне Горе, први пут је испитан због микрохематурије у петој години живота. Доказана је хиперкалциурија због које је лечен хидрохлортиазидом. После 12 година поново се јавио због макрохематурије и левостране нефролитијазе. Доказана је интермитентна хиперкалцемија, низак ниво паратхормона (PTH), хиперкалциурија и повећан ниво 25-хидрокси витамина Д [25(OH)$_2$D$_3$] у плазми. Болесник није узимао саплементе са витамином Д и сви познати узроци хиперкалцемије су искључени. Фамилијарна историја је позитивна за нефролитијазу (оба родитеља и бака по оцу), а сличне биохемијске абнормалности код оца и сина су указали на наследан поремећај. Откривена је хомозиготна мутација *CYP24A1* (*E143del*) код болесника и његовог оца, док је мајка била хетерозигот. У току даљег праћења од две године болесник је лечен екстракорпоралном литотрипсијом у четири наврата, повећаним уносом течности и избегавањем сунчања. На крају праћења он је без симптома, нормалне глобалне функције бубрега, нормалног ултрасонографског налаза уринарног тракта, али са хиперкалциуријом и ниским нивоом PTH у плазми.

Закључак Код болесника који имају идиопатску хиперкалциурију, нефролитијазу и нефрокалцинозу непознатог узрока, треба испитати витамин Д. Код мутације *CYP24A1* озбиљне компликације могу се избећи једноставним мерама: избегавање сунчања и витамина Д у витаминским саплементима и храни.

Кључне речи: Хередитарна нефролитијаза, нефрокалциноза, хипервитаминоза Д, идиопатска хиперкалцемија

INTRODUCTION

Metabolic disorders are a common cause of nephrolithiasis in childhood [1]. Of these, the most common is hypercalciuria which is found in 30-50% of the children with stones in the urinary system [2]. Hypercalciuria may be associated with normal, increased or decreased calcium in blood (Table 1). Hypercalcemic hypercalciuria may be found in hyperparathyroidism, but also in long-lasting immobilization, sarcoidosis, malignancy, juvenile idiopathic arthritis, corticosteroid excess, adrenal failure, William's syndrome and vitamin D hypervitaminosis.

Table 1. The causes of hypercalciuria.

HEREDITARY HYPERCALCIURIA	ACQUIRED HYPERCALCIURIA
Normocalcemic hypercalciuria	
Idiopathic	Prematurity
Dent's disease	Drugs: diuretics (furosemide, and acetazolamide), anticonvulsant use (topiramate, zonisamide), ketogenic diet
Antenatal Bartter syndrome	
Familial hypomagnesemia and nephrocalcinosis with hypercalciuria	
Distal renal tubular acidosis	
Hereditary hypophosphatemic rickets with hypercalciuria	
Hypocalcemic hypercalciuria	
Hypoparathyroidism	
Autosomal dominant hypocalcemic hypercalciuria	
Hypercalcemic hypercalciuria	
Primary hyperparathyreoidism (PHPT)	PHPT sporadic: Single parathyroid adenoma, not inherited
MEN1 syndrome associted PHPT	Long-lasting immobilization
Familial isolated PHPT	Sarcoidosis
Hyperparathyreoidism 2	Malignancies
Metaphyseal chondrodysplasia Jansen type	Juvenile idiopathic arthritis
Inherited hypophosphatasia	Corticosteroid excess
	Adrenal failure
	Vitamin D/vitamin A intoxication
Williams Beuren syndrome	Chronic kidney disease
Vitamin D induced infantile hypercalcemia-CYP24A1 gene mutation	Drugs: calcium carbonate, lithium

Historically, vitamin D hypervitaminosis was mainly attributed to vitamin D intoxication [3, 4] and/or to an intrinsic hypersensitivity to vitamin D [5]. However, with advancing in molecular examination of vitamin D metabolism our understanding of vitamin D hypervitaminosis significantly improved [6, 7]. Hypersensitivity to vitamin D, which has been observed in earlier studies [4, 5], now may be explained by the existence of a gene mutation leading to defective metabolization of active vitamin D [6]. Indeed, mutations of the vitamin D 24-hydroxylase (*CYP24A1*) which normally breaks down both $1,25(OH)_2D_3$ and $25(OH)D_3$ results in excessive formation of $1,25(OH)_2D_3$ [7]. Increased $1,25(OH)_2D_3$ levels cause hypercalcemia due to enhanced intestinal calcium absorption and hypercalciuria, because of reduced parathyroid hormone (PTH) -dependent calcium reabsorption in the distal renal tubule [7]. Affected individuals have hypercalcemia and hypercalciuria due to which they are prone to nephrolithiasis/nephrocalcinosis and consequently renal failure may occur. Being autosomal recessive inherited this genetic disorder often shows familial occurrence with increased risk in siblings who may experience the first clinical signs and diagnosis only in adulthood [8].

It is not rare that nephrolithiasis and nephrocalcinosis caused by *CYP24A1* mutations remain unrecognized despite extensive classical evaluation.

The aim of our work is to draw attention to hypercalciuria and nephrolithiasis caused by *CYP24A1* gene defects.

CASE REPORT

We report a male Montenegro patient who had primarily presented with microhematuria due to idiopathic hypercalciuria at 5 years of age. Renal ultrasound was normal, and he was treated by hydrochlorothiazide. After 12 years the patient presented with macrohematuria and left sided renal colic due to nephrolithiasis (Figure 1). He was found to have intermittent borderline hypercalcaemia (serum Ca 2.46-2.66 mmol/l), low level of intact parathyroid hormone (PTH) (<0.26 pmol/l), hypercalciuria (11.6 mmol/24 h) and increased plasma 25-hydroxy vitamin D [25(OH)D_3] (137.3 nmol/l). Serum 1,25$(OH)_2D_3$ was not measured. The patient denied using vitamin D supplementation, but certainly had a lot of seasonal sunlight exposure due to Mediterranean climate. Serum electrolytes including magnesium and phosphorus were normal as well as serum bicarbonate, urea and creatinine. Twenty-four-hour urine evaluations excluded hyperuricosuria and oxaluria. Also, other causes of hypercalcemia were ruled out. Chemical analysis of stone found calcium oxalate.

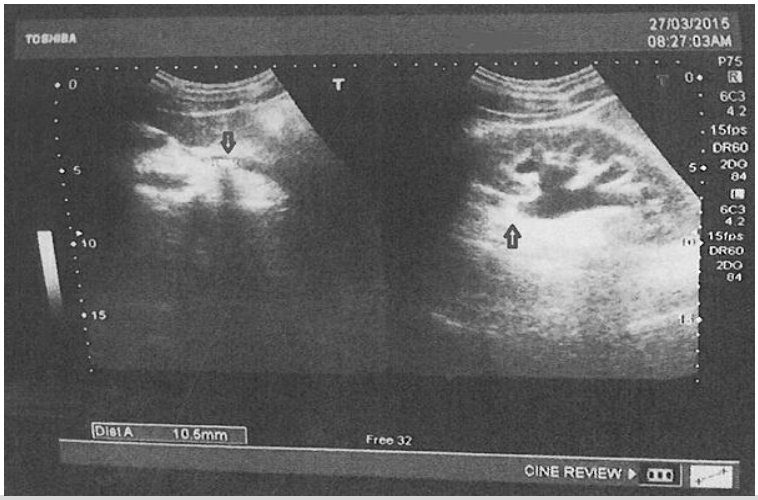

Figure1. Renal ultrasound displaying the patient´s left kidney: hydronephrosis due to stone in proximal part of the left ureter (arrowed).

During further follow-up of two years he was treated by four courses of extracorporeal shockwave lithotripsy, increased water intake, and he was advised to avoid sunlight exposure. At the end of follow-up he was asymptomatic, and his renal ultrasound was normal, as well as his renal function. The latest biochemical findings were as follows: serum calcium normal (2.34 mmol/l; Ca^{++} 1.12 mmol/l), intact PTH low (1.37 pmol/l), 25(OH) D_3 in the upper normal range (123.5 nmol/l) and increased 24 hour calciuria (8.88 mmol/24 h).

The patient's family history was positive for kidney stones: in father (at age of 17 years), mother (at age of 35 years) and the paternal grandmother. At the time of this study, renal ultrasound was normal in both parents, but hypercalcemia (2.62 mmol/l), hypercalciuria (12.41 mmol/24h), depressed PTH (1.07 pmol/l) and increased 25(OH) D_3 (94.3 nmol/l) were found in father as well as in his son. Familial occurrence of nephrolithiasis pointed-out its inherited occurrence. Using PCR and Sanger sequencing, a homozygous mutation in *CYP24A1* (E143del) was found in patient and his father, while mother is heterozygous. The parents declared not to be consanguineous.

DISCUSSION

Our patient as well as his father has E143del homozygous mutation in CYP24A1. This mutation, previously described by Schlingmann et al. [9], leads to a complete loss of 25-OH-D3-24-hydroxylase activity that results in persistently increased levels of both $1,25(OH)_2D_3$ and $25\ (OH)D_3$ and the absence of any measurable inactive metabolite. Basal renal and extrarenal CYP24A1 is usually low but is highly induced by its substrate $1,25(OH)_2D_3$.

In regulating the level of vitamin D3, CYP24A1 plays a role in calcium homeostasis and the vitamin D endocrine system. Its expression is highest in the intestine, kidney and the skin where this enzyme acts to remove metabolites of vitamin D [10]. It has been demonstrated that CYP24A1 knockout (–/–) mice suffer from increased sensitivity to exogenous vitamin D intake and approximately half of them die due to severe hypercalcemia [11]. In humans, *CYP24A1* mutations can cause idiopathic infantile hypercalcemia (IIH) [12-19], idiopathic hypercalciuria [9], nephrocalcinosis, and possibly reduced bone density [20]. In patients with IHH due to *CYP24A1* mutations, even small doses of vitamin D, as prescribed for vitamin D prophylaxis, may provoke symptomatic hypercalcemic crisis which need treatment by acute hemodiafiltration [16]. Increased sensitivity to vitamin D in patients with *CYP24A1* mutations has been also documented by seasonal variations of vitamin D and calcium parameters due to sunlight exposure [17, 18]. Calcemia may be influenced also by alimentary factors. Those may explain the intermittent character of hypercalcemia in our patient too as he did not receive any vitamin D supplement. During his first clinical examination at five years of age it was a winter time, and investigation did not reveal hypercalcemia, but only hypercalciuria. Therefore, in patients with idiopathic hypercalciuria, serum calcium level should be monitor carefully throughout life.

Kidney damage may occur in patients with *CYP24A1* mutations, because of nephrolithiasis and / or nephrocalcinosis. It has been estimated that the overall frequency of kidney stones due to CYP24A1 deficiency is 4%–20% [20, 21]. However, it probably may be even higher in children as the majority of children with nephrolithiasis have a metabolic background and familial occurrence [1]. Our patient had familial history of nephrolithiasis. His father, who has the identical CYP24A1 mutation and almost the identical biochemical alterations, had kidney stone at adolescent age, but with milder clinical course. It is uncertain just that transient nephrolithiasis in patient's mother was the consequence of the heterozygous *CYP24A1* mutation. Data from literature suggest that most heterozygous *CYP24A1* mutation carriers have a normal vitamin D, usually are asymptomatic, but may possibly be at increased risk of nephrolithiasis [22].

Treatment options for *CYP24A1* mutation disorders include avoidance of vitamin D supplementation, sunlight exposure and tanning beds and high volume intake, while in severely affected patients, treatment with the cytochrome inhibitor ketoconazole may be beneficial [23].

ACKNOWLEDGMENT

Writing this paper was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia, grant No. 175079.

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