PURINE DISORDERS WITH HYPOURICEMIA

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Abstract

Hypouricemia is defined as a serum urate levels less than 2 mg/dL (119 μmol/L). Primary hypo-uricemia is caused by disorders of purine metabolism and transport. This laboratory finding is some-times overlooked and, following two genetic defects, should be considered in differential diagnosis of unexplained hypouricemia. Hereditary xanthinuria is autosomal recessive and due to mutations in xanthine oxidase, leading to over-production of xanthine and minimal production of urate. Patients have very low serum urate levels and suffer from elevated levels of xanthine in the urine, leading to xanthine stones, haematuria, and sometimes occult chronic kidney failure. Hypouricemia is the key to diagnosis. Hereditary renal hypouricemia is a new genetic defect of renal transport of uric acid. Two types were distinguished: a) renal hypouricemia type 1, caused by the defects in the *SLC22A12* gene coding the human urate transporter 1 (hURAT1) and b) renal hypouricemia type 2, caused by the defects in the *SLC2A9* gene, which encodes GLUT9 transporter. This disorder predisposes pati-ents to exercise-induced acute renal failure and/or nephrolithiasis. Diagnosis is based on two mar-kers: hypouricemia (< 119 μmol/L) and increased fractional excretion of uric acid ($> 10\%$). Over one hundred cases were identified in Japan and and this number is unique worldwide. Several patients were described in Macedonia. We were able to detect four Czech families with hereditary xanthi-nuria and eight cases of hereditary renal hypouricemia. In conclusion, hereditary xanthinuria and hereditary renal hypouricemia are still unrecognized conditions. Patients with unexplained hypouri-cemia need detailed purine metabolic investigations.

Key words: hypouricemia, purine metabolism, hURAT1, GLUT9.

Introduction

Genetic defects of purine metabolism cover a broad spectrum of illnesses. They represent a group of relatively new disorders. The first ge-netic purine disorder – xanthinuria, was descri-bed as the cause of renal calculi in 1954 [1]. The genetic basis for Lesch-Nyhan syndrome with juvenile gout and severe neurological im-pairment was discovered in 1967 [2]. The num-ber of inborn errors of purine and pyrimidine metabolism has increased since then and now totals 27. The diagnostic problem is compoun-ded by a limited awareness due to the relatively recent description. This paper describes defects with hypouricemia.

Uric acid, hypouricemia

Uric acid (2, 6, 8-trihydroxypurine) is the end product of purine metabolism in primates, including man. This metabolite is formed from the precursor purine bases xanthine and hypo-xanthine by the action of xanthine oxidase. The name purine was given to this important group of heterocyclic compounds by Fisher in 1899.

Professor of Medical Chemistry at the Czech Medical Faculty of Charles University, Prague, Jan Horbaczewsky was the first who carried out the synthesis of uric acid. He performed his studies in the 1880s and 1890s [3]. A lack of the enzyme uricase allows this poorly soluble substance to accumulate in body fluids in man. In most other mammals it is futher metabolized to the more soluble allantoin. It is because of the poor solubility of urates that humans are predisposed to clinical gout and renal damage by high levels of uric acid. Uric acid contains two dissociable protons, but only the first is important in biological fluids. At physiological pH of blood only the hydroxyl group at the eight position, which has a pK of 5.4, dissociates. So in plasma pH 7.4, about 98% of uric acid exists in the form of monosodium salt. However, in acid urine, a substantial amount may be undissociated. The undissociated acid is often less soluble in water than sodium urate.

The uric acid concentrations in serum vary with age and sex. Children of both sexes have a serum urate concentration of 180 to 240 μmol/L. Only boys at puberty exhibit a further elevation of 60 to 120 μmol/L, which is generally sustained through life. Serum uric acid concentration in women at menopause rises and approaches the values for adult males [2].

Solutions of monosodium urate become supersaturated when concentration exceeds 420 μmol/L. However, the relationship between the presence and severity of hyperuricemia and the development of gouty arthritis or renal calculi is more complex than simple considerations of solubility might suggest.

Renal excretion of urate is a complex process. Under normal conditions, only trace amounts of purine bases and nucleotides are present in the urine. Two-thirds of daily urate production is excreted by the kidney, the rest by the gastrointestinal tract. Except for a small fraction bound to plasma proteins, urate is completely filtered at the glomerulus. This is then mostly reabsorbed in the proximal tubule. The reabsorption is higher in males (92%) than in females (88%) and is lower in children of both sexes (70–85%). This probably explains the rare incidence of gout in women and children [2]. There are numerous urate transporters in the proximal tubule, resulting in both secretion and reabsorption by different transport mechanisms [4], and many of these transporters are involved also in the transport of other organic anions [4, 5]. The uric acid pool in humans is determinated by the balance between synthesis and excretion. Production can be increased by several mechanisms, including genetic defects and states of high cell turnover and alcohol ingestion; however, it is important to stress that the majority of cases with elevated serum uric acid levels result from impaired renal excretion [4]. Whereas hyperuricemia is a metabolic risk factor and is capable of causing disease by itself, hypouricemia may indicate an underlying pathological condition. Hypouricemia is defined as a serum urate level less than 2 mg/dL (119 μmol/L). The prevalence in the general population is 0.2% and in hospitalized patients is 1.2% [6]. Primary hypouricemia is caused by disorders of purine metabolism and transport. This laboratory finding is sometimes overlooked and following two genetic defects should be considered in differential diagnosis of unexplained hypouricemia.

Hereditary xanthinuria

This genetic defect of purine metabolism results from a deficiency of xanthine dehydrogenase (XDH) with an autosomal recessive mode of inheritance. Hereditary xantinuria is classified into three categories. In type I, only xanthine dehydrogenase is lacking. In type II, in addition, aldehyde oxidase activity is also deficient. A third type, molybdenum cofactor deficiency is characterized by the lack of sulphite oxidase deficiency, as well as xanthine dehydrogenase and aldehyde oxidase activities. All types are characterized by very low or even undetectable concentrations of urate in blood and urine and a very high concentration of xanthine in urine (more then 25 μmol/mol creatinine). Hypouricemia is the key to diagnosis. More than 50% of patients remain asymptomatic. The incidence of this genetic defect is unknown. So far, approximately 150 cases have been described worldwide. An annual incidence has been estimated between 1 : 6,000 and 1 : 69,000. This wide variability is due to the fact that half of the affected individuals remains asymptomatic and therefore this condition is underdiagnosed. In addition, newborn screening for this genetic

defect is not performed and so the precise incidence determination is not available. Therefore reported numbers of incidence are just rough estimates. The disorder appears to be relatively prevalent in the Mediterranean region [7, 8]. Symptom onset may be at any age. Approximately 50% of patients with classical xanthinuria present with symptoms of urinary tract infection, haematuria, renal colic, acute renal failure, crystalluria or urolithiasis. In some rare patients, renal disease may evolve to kidney failure, or may even induce arthropathy, myopathy or duodenal ulcer. Diagnosis is based on estimation of uric acid in blood and urine. If hypouricemia is found, detailed purine metabolic investigation follows and includes the measurement of xanthine and hypoxanthine in urine and plasma. High urinary levels of xanthine are then very typical for classical xanthinuria. Additional methods for diagnosis confirmation and/or identification of the type of xanthinuria include the allopurinol loading test, xanthine oxidase assay and molecular analysis [9–11]. In therapy, low purine diet and high fluid intake is recommended. Since the solubility of xanthine is not affected by urinary pH, alkalization is of no value. When calculi are present, a pyelolithotomy might be necessary [11].

Primary hereditary renal hypouricemia

This disorder is a new genetic defect of renal transport of uric acid. Two types were distinguished: a) renal hypouricemia type 1, caused by the defects in the *SLC22A12* gene coding the human urate transporter 1 (hURAT1) and b) renal hypouricemia type 2, caused by the defects in the *SLC2A9* gene, which encodes GLUT9 transporter. The human urate transporter 1 (hURAT1) acts as an influx transporter for urate at the apical membrane at the proximal renal tubule. GLUT9 is an efflux transporter, transporting urate from tubular cell to interstitium /blood space. This disorder predisposes patients to exercise-induced acute renal failure and/or nephrolithiasis. Approximately 50% of patients remain asymptomatic. Haematuria is sometimes only present [12]. Over one hundred cases were identified in Japan and and this number is unique worldwide [12, 13]. Several patients were described in Macedonia [14]. Diagnosis is based on two markers: hypouricemia (< 119 μmol/L) and increased fractional excretion of uric acid ($> 10\%$). Confirmation of diagnosis is done by molecular analysis of *SCL22A12* and *SLC2A9* genes [11, 15, 16]. Therapy is based on high fluid intake, alkalization of urine and and avoidance of strenuous exercise [12]. Clinicians who have questions regarding diagnosis of this condition should feel free to contact the first author, who is interested in the characterization of this disorder and is able to provide complete investigations for confirmation of diagnosis. For patients in whom no mutation has been found, referral to an academic medical centre will help further research.

Materials and methods

Over the last 3 years more than 570 samples referred to our department with unexplained hypouricemia and suspicious of purine genetic defect were investigated. In suspected cases detailed purine metabolic investigation was performed.

Serum and urinary uric acid was measured by specific enzymatic method. Creatinine was measured by standard rate-dependent Jaffe based methods. Endogenous purine metabolites were investigated in lysed erythrocytes as described [17]. The xanthine oxidase activity was determined using a modified method published previously [18].

Results

We were able to detect our first patients with hereditary xanthinuria in our Czech population.

Table 1 summarizes biochemical findings in three patients of Czech origin. The parents of case one were also investigated. The age range of the patients was between 9 and 43 years. Two biochemical markers are evident. Uric acid in serum was very low (15–16 μmol/L) or even undetectable. The second marker – levels of urinary xanthine – were very high in the range of 170–327 mmol/mol creatinine, in contrast to lower concentrations in the healthy father and mother of case 1. In addition, in two patients

and the parents of patients one, xanthine dehydrogenase activity in plasma was measured [19]. Low or even undetectable activity was found. All these findings confirmed a diagnosis of hereditary xanthinuria.

In addition, we were able to detect our first patients with primary renal hypouricemia as described previously [15, 16, 20].

Table 1

Biochemical findings in patients with XDH deficiency

Case, age	Uric acid in serum (μmol/l)	Xanthine in urine (mmol/molCr)	Xanthine dehydrogenase activity plasma (pmol/h/ml)
1 43	15	190	0,37
2 29	not detected	170	0,00
Father 48	268	18	3,35
Mother 48	182	14	8,38
3 9	16	327	not performed
Reference	120–340	< 25	3,2–9,2
Range	120–416		

Discussion

The diagnostic problem of genetic defects of purine metabolism is compounded by a limited awareness due to their relatively recent description. From the diagnostic point of view, there is an advantage for the two of these disorders, in which hypouricemia is the first biochemical sign. Hypouricemia has been proved in hereditary xanthinuria and primary renal hypouricemia as a good biochemical marker [4, 12], so measurement of urate levels in blood and urine in suspicious patients is very important [11]. In addition, the importance of hyperuricemia also arises because of its potential to cause not only gout but also hypertension and renal disease. Recent studies have found that soluble uric acid has proinflammatory and proliferative effects on vascular smooth muscle cells and causes dysfunction of endothelial cells. Uric acid has thus a role as a true risk factor for kidney disease [21].

Our experience in the detection of genetic defects of purine metabolism shows that the finding of hypouricemia needs further investigation in specialized laboratories in order to exclude other secondary causes of hypouricemia. These include conditions such as Fancony syndrome, Wilson disease, cystinosis, heavy metal poisoning, liver diseases, medication with uricosuric agents, etc. A third type of xanthinuria (molybdenum cofactor deficiency) with combined xanthine dehydrogenase/aldehyde oxidase/sulphite oxidase deficiencies is associated with neurological symptoms such as dysmorphic features, seizures (often refractory to anticonvulsants), mental retardation and elevated concentrations of sulfocysteine in urine [22]. The diagnosis required multidisciplinary approach. Currently there are few laboratories in Europe providing the necessary diagnostic service. We were able to find our first patients with hereditary xanthinuria in our Czech population. Two biochemical markers (hypouricemia less than 120 μmol/L and elevation of urinary xanthine more than 25 μmol/mol creatinine) were evident in all patients and were sufficient for the establishment of diagnosis. In addition, measurement of xanthine dehydrogenase activity in plasma confirmed diagnosis. Our experience shows that even grossly elevated urinary xanthine (327 μmol/L) in case No. 3 did not contribute to the formation of xanthine stones.

All our patients with primary renal hypouricemia had profound hypouricemia and this finding was the first reason for referral of these suspected patients to our department [15, 16, 20].

In conclusion, hereditary xanthinuria and hereditary renal hypouricemia are still unrecognized conditions. Patients with unexplained hypouricemia need detailed purine metabolic investigation.

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Р е з и м е

ПУРИНСКИ НАРУШУВАЊА СО ХИПОУРИКЕМИЈА

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Хипоурикемија се дефинира како вредности на серумски урат помали од 2 mg/dL (119 µmol/L). Примарната хипоурикемија е предизвикана од заболувањата на пуринскиот метаболизам и транспорт. Овој лабораториски наод кој

некогаш се превидува и во однос на двата генски дефекта би требало да се разгледа во диференцијална дијагноза на необјаснета хипоурикемија. Хередитарна ксантинурија е автозомно рецесивна болест и се должи на мутации во ксантин оксидазата, што води до хиперпродукција на ксантин и минимална продукција на урат. Пациентите имаат многу ниски вредности на серумски урат и страдаат од зголемени вредности на ксантин во урината, што води до појава на ксантински камења, хематурија и понекогаш окултна хронична бубрежна слабост. Хипоурикемијата е клуч кон дијагнозата. Хередитарна ренална хипоурикемија е нов генетски дефект во бубрежниот транспорт на уринечната киселина. Се разликуваат два типа: а) ренална хипоурикемија тип 1 предизвикана од дефектот во *SLC22A12* генот, кој го кодира хуманиот уратен транспортер 1 (hURAT1) и б) ренална хипоурикемија тип 2 предизвикана од дефектот во *SLC2A9* генот, кој го кодира GLUT9 транспортерот. Пациенти со овие нарушувања се предиспонирани да развијат акутна бубрежна слабост по физички напор и/или нефролитијаза. Дијагнозата се базира на 2 маркера: хипоурикемија (< 119 µmol/L) и зголемена фракциона екскреција на уринечната киселина (> 10%). Повеќе од 100 случаи се опишани во Јапонија и во светски размери ова е уникатна бројка. Неколку пациенти, исто така, се опишани во Македонија. Ние успеавме да детектираме 4 чешки фамилии со хередитарна ксантинурија и осум случаи на ренална хипоурикемија. Во заклучок, хередитарната ксантинурија и хередитарната ренална хипоурикемија сѐ уште се непрепознаени кондиции. Пациенти со необјаснета хипоурикемија бараат детална процена на пуринскиот метаболизам.

Клучни зборови: хипоурикемија, пурински метаболизам, hURAT1, GLUT9.