CASE REPORT

Intrahepatic Cholestasis due to Citrin Deficiency

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Abstract

We report a case of neonatal intrahepatic cholestasis due to citrin deficiency in a 2-month-old female of Chinese descent who presented with jaundice and acholic stools. She had poor weight gain, axial hypotonia, and a large anterior fontanelle with no dysmorphia or hepatosplenomegaly. Laboratory findings showed cholestasis, elevated transaminases, hypoalbuminemia, prolonged prothrombin time, anemia and galactosuria. Obstructive, infectious, and genetic causes (Alagille syndrome, alpha-1-antitrypsin deficiency, cystic fibrosis) were excluded as well as galactosemia. Plasma amino acids chromatography exhibited elevated citrulline, tyrosine, methionine, and threonine, raising a strong suspicion of citrin deficiency. The patient was started on a lactose-free formula with medium-chain triglycerides and fat-soluble vitamins, with rapid clinical and laboratory improvement. Genetic analysis confirmed compound heterozygosity in the SLC25A13 gene.

We emphasize the importance of considering this hypothesis in the differential diagnosis of neonatal cholestasis, especially in patients of Asian origin, given the possibility of therapeutic intervention and prevention of complications.

Keywords: Cholestasis, Intrahepatic/etiology; Citrullinemia/etiology; Citrullinemia/diet therapy; Citrullinemia/genetics; Diagnosis, Differential; Infant

Introduction

Cholestasis affects approximately one in every 2,500 term infants.[1] The differential diagnosis is of great importance since it includes potentially severe but treatable diseases. Etiologies can be grouped as extra-hepatic (*e.g.* obstructive) and intra-hepatic (including infectious, metabolic, genetic, endocrine, or alloimmune causes).[2]

In a large systematic review, the most common specific etiologies were biliary atresia (25.89%), infection (11.47%), total parenteral nutrition-associated cholestasis (6.44%), metabolic disease (4.37%), alpha-1 anti-trypsin deficiency (4.14%), and perinatal hypoxia/ischemia (3.66%). Idiopathic neonatal hepatitis occurred in 26.0% of cases.[3]

The number of patients with idiopathic neonatal hepatitis is declining alongside the advances in diagnostic evaluation and the identification of new etiologies, namely with the use of the available next-generation deoxyribonucleic acid (DNA) sequencing technologies.[4]

Citrin deficiency (CD) is a newly recognized disease with a variable phenotype. In newborns and infants, it presents as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in older children as a failure to thrive and dyslipidemia caused by citrin deficiency, and in adults as recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2).[5] Severity can vary significantly, from spontaneous resolution in NICCD to liver failure in CTLN2.

The disease is caused by a biallelic mutation of the *SLC25A13* gene that codifies a mitochondrial transporter aspartate/glutamate carrier (AGC), named citrin.[6] Aspartate/glutamate carrier 2 (AGC2) is the only isoform in the liver, and is responsible for the transport of aspartate from the mitochondria to the cytosol in the urea cycle, and the transport of nicotinamide adenine dinucleotide bonded with hydrogen (NADH) reducing equivalent from the cytosol into the mitochondria, as a member of the malate-aspartate shuttle (Fig. 1).[7] Therefore, AGC2 deficiency generates an increase in the cytosolic NADH/NAD ratio that impairs glycolysis, gluconeogenesis, and galactose metabolism, along with a decrease in cytosolic aspartate that interferes with arginosuccinate synthase activity that leads to citrulline accumulation and hyperammonemia.[8]

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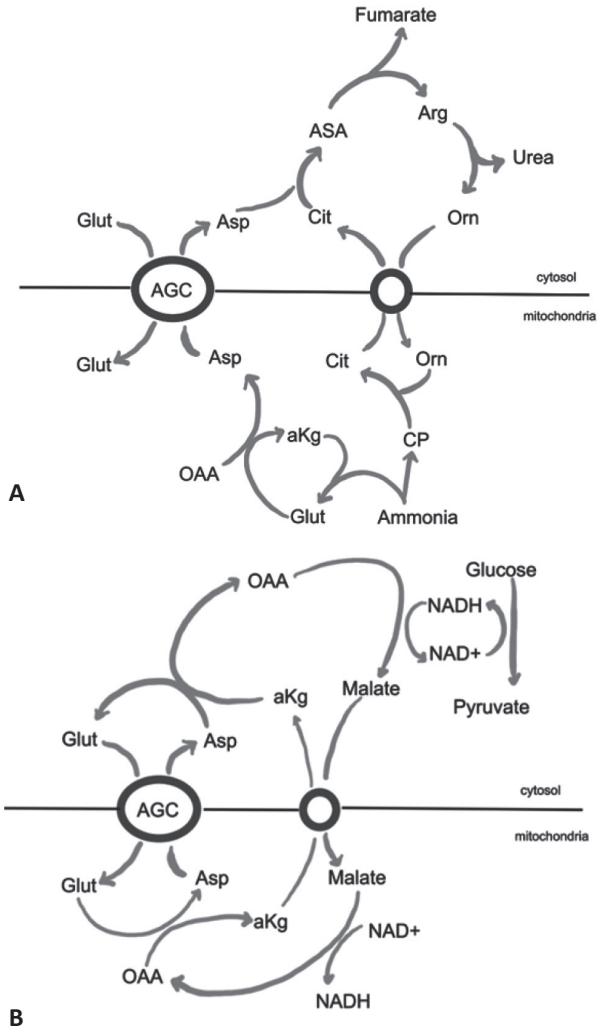
Case Report

A 2-month-old girl was brought to the emergency department with a one-day history of jaundice and acholic stools. She was the second child of non-consanguineous Chinese parents, born at full term, with prenatal growth restriction, low birth weight, and was being breastfed.

Physical examination showed a well-appearing infant, below the third percentile for weight and length, with generalized jaundice, hypotonia, and large anterior fontanelle, but otherwise a normal morphologic and neurological examination. Hepatosplenomegaly and heart murmur were absent. Laboratory data exhibited conjugated hyperbilirubinemia with total bilirubin (TB) 10.6 mg/dL, conjugated bilirubin (CB) 7.5 mg/dL), alanine aminotransferase (ALT) 77 U/L, aspartate aminotransferase (AST) 224 U/L, alkaline phosphatase (AP) 1348 U/L, gamma glutamyl transpeptidase (GGT) 327 U/L, prothrombin time (PT) 19.1 s, international normalized ratio (INR) 1.7, glucose 57 mg/dL, albumin 29 g/L, fibrinogen 1 g/L, factor V 144%, factor VII 53%, hemoglobin 8.3 g/L, white blood cells 15,450 cells/µL, platelets 614,000 cells/µL. Renal function, ionogram, ammonia, and total cholesterol were normal. Lactate was slightly increased (3.2 mmol/L).



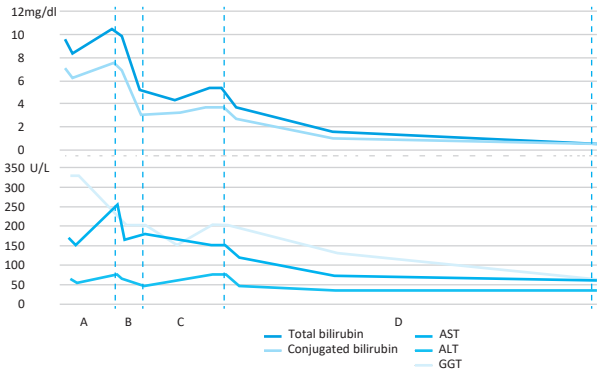
AGC - aspartate glutamate carrier; aKg - a-ketoglutarate; Arg - arginine; ASA - argininosuccinate; Asp - aspartate; CP - carbamoylphosphate; Cit - citrulline; Fum - fumarate; Glut - glutamate; H - hydrogen; Mal - malate; NAD - nicotinamide adenine dinucleotide; OAA - oxaloacetate; OMC - oxoglutarate malate carrier; Orn - ornithine.
Adapted from: Saheki T, et al. Adult-onset type II citrullinemia and idiopathic neonatal hepatitis caused by citrin deficiency: Involvement of the aspartate glutamate carrier for urea synthesis and maintenance of the urea cycle. Mol Genet Metab 2004;81:S20-6. doi: 10.1016/j.ymgme.2004.01.006.[7]

Figure 1. Aspartate glutamate transporter in (A) urea cycle and (B) malate-aspartate shuttle.

The abdominal ultrasound showed a normal liver and splenic parenchyma and no signs of biliary obstruction. She was started on ursodeoxycholic acid, vitamin K, and fat-soluble vitamins and was admitted for investigation.

Since she displayed intermittent acholic stools, low GGT serum levels, and normal abdominal ultrasound, biliary atresia was unlikely.

She had no evidence of TORCH group infections (toxoplasmosis, rubella, cytomegalovirus, *herpes simplex*, other agents). Ophthalmological, cardiac, and renal examinations as well as vertebral morphology and serum alpha-1-antitrypsin were normal. The newborn screening for cystic fibrosis, congenital hypothyroidism, and metabolic diseases (including tyrosinemia) was also negative. She had slightly increased levels of serum galactose in the newborn screening sample (6.6 mg/dL, upper normal limit 5.5 mg/dL). Reducing substances in urine were positive and sugar chromatography revealed raised levels of galactose, once more. Considering the possibility of classic galactosemia, while waiting for the results of erythrocyte galactose-1-phosphate (G-1-P) and galactose-1-phosphate uridyltransferase (GALT), breastfeeding was suspended, and she started on a lactose-free formula. As shown in Fig. 2, this change was associated with a progressive decrease in TB (5.09 mg/dL) and liver enzymes (AST 167 U/L, ALT 63 U/L, GGT 150 U/L, AP 683 U/L). Later on, as the G-1-P level (24,8 µmol/L, reference values 7-22 µmol/L) and GALT activity (724.3 µmol/L/mmol Hb/h, reference values 300-800 µmol/L/mmol Hb/h) excluded classic galactosemia, breastfeeding was reintroduced. However, liver enzymes and TB raised again (AST 180 U/L, ALT 76 U/L, GGT 202 U/L, AP 1267 U/L, TB 5.36 mg/dL, CB 3.7 mg/dL) (Fig. 2). Plasma amino acids chromatography showed high citrulline (80 µmol/L, reference values 0-10 µmol/L), threonine (403 µmol/L, reference values 17-92 µmol/L), methionine (274 µmol/L, reference values 6-22 µmol/L), and tyrosine (206 µmol/L, reference values 12-52 µmol/L), which was highly suggestive of citrin deficiency. After these results, the patient restarted the lactose-free formula with a medium-chain triglycerides



ALT - alanine aminotransferase; AST - aspartate aminotransferase; GGT - gamma glutamyl transpeptidase.
Phases: A - breastfeeding; B - lactose free formula; C - breastfeeding reintroduction; D - lactose free formula with medium chain triglyceride supplementation.

Figure 2. Total and conjugated bilirubin, alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transpeptidase levels evolution according to time and diet.

supplementation that resulted in a decrease of TB (4.35 mg/dL), CB (3.21 mg/dL), and liver enzymes (AST 162 U/L, ALT 63 U/L, ALP 771 U/L, GGT 154 U/L) (Fig. 2) after which she was discharged.

Two weeks later, she had further improvement of the liver tests with TB 1.52 mg/dL, CB 1.03 mg/dL, ALT 38 U/L, AST 72 U/L, GGT 132 U/L, AP 439 U/L (Fig. 2). At the age of 6 months, she showed normal psychomotor development, weight, and length gain, no urinary reducing substances and normal plasma threonine (72 µmol/L, reference values 114-335 µmol/L), methionine (41 µmol/L, reference values 9-41 µmol/L), and tyrosine (82 µmol/L, reference values 42-99 µmol/L) although still slightly elevated citrulline (55 µmol/L, reference values 15-30 µmol/L). At this time, she started complementary feeding with a protein and lipid rich diet, with carbohydrate and lactose restriction.

SLC25A13 gene sequencing showed two variants in heterozygosity, a pathogenic variant *c852-855delTATG* (*p.Met285Profs*2*), common in Chinese, Japanese, and Korean populations and a variant of uncertain clinical significance (*c.1067G>A*). Both parents are carriers.

Discussion

Until recently, citrin deficiency was thought to be restricted to Japan but now it is recognized to be panethnic.[9] In Japan, the frequency of homozygotes or compound heterozygotes for *SLC25A13* pathogenic variants is calculated at 1:17,000 based on the carrier (heterozygote) rate of 1:65.[10] In continental China, the carrier frequency varies geographically from south (1:48) to north (1:948) of the Yangtze river.[11] These infant parents are from Fujian, south of the Yangtze.

Neonatal intrahepatic cholestasis caused by citrin deficiency occurs in infants and presents with low birth weight with growth restriction and transient intrahepatic cholestasis, hepatomegaly, variable liver dysfunction, hypoproteinemia, and/or hypoglycemia.[5]

The diagnosis is established with the characteristic biochemical findings (increased plasma citrulline and arginine and high threonine/serine ratio) and identification of biallelic pathogenic variants in *SLC25A13*.[5] All these features were present in our case.

Noteworthy, NICCD infants can have a false positive newborn screening for citrullinemia or classic galactosemia but follow up diagnostic tests will not confirm these diseases.[5] Furthermore, some patients, like this one, miss detection by universal newborn screening using tandem mass spectrometry.

Neonatal intrahepatic cholestasis caused by citrin deficiency is generally not severe and symptoms usually resolve by 1 year of age with treatment, including lactose (galactose)-free and medium-chain triglyceride fat therapeutic formulas,[12] soluble vitamin supplementation, along with low-carbohydrate, and high-protein/-fat diets.[8] Risk factors associated with mortality in NICCD cases are late referral, presence of infection, delayed treatment with lactose (galactose)-free and medium-chain triglyceride fat therapeutic formulas, lower platelet count, lower levels of GGT, total cholesterol and blood citrulline, and higher level of blood ammonia and tyrosine.[13] Our patient followed the dietetic plan with the rapid improvement of the clinical and metabolic condition. None of the poor prognosis risk factors were present, although liver dysfunction with low albumin, hypoglycemia, and high INR were observed at presentation.

It is advisable to identify the affected siblings of a proband so that appropriate dietary management can be instituted before symptoms occur.[5] In this case, we plan to study the older brother especially because CTLN2 is more serious and more common in males and might present at an older age without the NICCD symptoms in the first year of life.[5]

Starting around age 1-2, children show a strong preference for protein-rich and lipid-rich foods and an aversion to sugar-rich and carbohydrate-rich foods.[14]

This adaptation/compensation stage is characterized by the various signs and symptoms, such as hypoglycemia, fatty liver, easy fatigability, weight loss, and neuropsychiatric symptoms.[15] Some poorly-controlled patients show failure to thrive and dyslipidemia caused by citrin deficiency.[15,16]

Even if asymptomatic and under a restrictive diet, biochemical changes usually persist, like dyslipidemia,

elevated lactate/pyruvate ratio, high citrulline and ornithine plasma levels as well as augmented oxidative stress markers.[17]

About one fifth of the affected children develop CTLN2 at ages of 11-79.[17] This potential fatal hyperammonemia encephalopathy is also associated with chronic pancreatitis, hepatocellular carcinoma (without cirrhosis and sometimes before CTLN2 manifestation), hyperlipidemia, and non-alcoholic fatty liver. Even though liver transplantation is the most effective treatment, once it is re-establishes normal hepatic metabolism,[8] dietary factors might influence evolution to CTLN2.[17]

There can be hyperammonemic crisis, precipitated by alcohol, high carbohydrate intake, drugs like acetaminophen or rabeprazole, infections, and surgeries.[5] Paradoxically to what happens in other urea cycle defects,[18] dextrose, fructose, or glycerol infusions should be avoided in crisis with brain edema and mannitol administration should be the first option.[8] Arginine supplementation is effective in preventing hyperammonemia and hypertriglyceridemia.[8] Sodium pyruvate seems to delay the need for liver transplantation,[8] although it does not seem to correct the tricarboxylic acid cycle deviation.[19]

Patients with NICCD should be followed closely for the early identification of poor prognosis cases.[20] After the first year of age, close surveillance of anthropometric indexes, serum lipid levels including triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol is appropriate[5] in order to early detect poorly controlled patients with impaired growth or hyperlipidemia.[15] Plasma ammonia (two hours after meals), citrullinemia, and trypsin secretion inhibitor should be monitored at least twice a year.[5] Increased levels might evidence progression to CTLN2,[21,22] and so treatment should be initiated promptly.[5]

WHAT THIS CASE REPORT ADDS

- In the current globalized world, it is important to consider a patient's origins, since there are important differences in the prevalence of many conditions, and it might help to establish the diagnosis as an important step to provide more adequate treatment to patients.
- In neonatal citrin deficiency, it is important to have a diagnosis not only to provide treatment but also to identify and follow up on the patients in order to prevent complications, such as failure to thrive and dyslipidemia during childhood and adolescence and hyperammonemia crisis in adulthood.

Conflicts of Interest

The authors declare that there were no conflicts of interest in conducting this work.

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Consent for publication was obtained.

Confidentiality of data

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

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Colestase Intrahepática por Deficiência de Citrina

Resumo

Relatamos um caso clínico de colestase intra-hepática neonatal por défice de citrina numa lactente de 2 meses de idade de origem chinesa, que se apresentou com icterícia e fezes acólicas. Apresentava também má evolução ponderal, hipotonia axial e uma fontanela anterior de grandes dimensões, sem dismorfia ou hepatoesplenomegalia. A avaliação laboratorial evidenciou colestase, elevação das transaminases, hipoalbuminemia, prolongamento do tempo de protrombina, anemia e galactosúria. Foram excluídas causas obstrutiva, infeciosa e genética (síndrome de Allagille, défice de alfa-1-antitripsina, fibrose quística), assim como galactosemia.

A cromatografia dos aminoácidos plasmáticos demonstrou elevação da citrulina, tirosina, metionina e treonina, sugerindo a hipótese diagnóstica de défice de citrina. A doente iniciou fórmula láctea sem lactose, suplementada com triglicéridos de cadeia média e vitaminas lipossolúveis, verificando-se uma rápida melhoria clínica e laboratorial. O estudo genético confirmou heterozigotia composta no gene *SLC25A13*. Destacamos a importância de considerar o défice de citrina no diagnóstico diferencial da colestase neonatal, principalmente em doentes de origem asiática, atendendo a possibilidade de intervenção terapêutica e prevenção de complicações futuras.

Palavras-Chave: Citrulinemia/dietoterapia; Citrulinemia/etiologia; Citrulinemia/genética; Colestase Intra-Hepática/etiologia; Diagnóstico Diferencial; Lactente