Urinary reducing substances in neonatal intrahepatic cholestasis caused by citrin deficiency

Ajmal Kader¹, Christina Ong¹, Veena Logarajah¹, Kong Boo Phua¹, Ee Shien Tan²

¹Gastroenterology Service, Department of Pediatric Medicine, KK Children’s and Women’s Hospital, Singapore
²Genetics Service, Department of Pediatric Medicine, KK Children’s and Women’s Hospital, Singapore

Abstract

Neonatal cholestasis due to citrin deficiency is an autosomal recessive metabolic disorder caused by mutations in SLC25A13 gene. Mutations in this gene have a relatively high prevalence in East-Asian races compared to European or Afro-Caribbean races. Mutations in both sets of chromosomes often lead to self-limiting early onset cholestasis and growth retardation referred as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD). It is associated with a wide range of metabolic derangements including galactosemia and aminoacidemia, which can be detected on the newborn blood spot screening. Galactose, being a reducing sugar, can also be detected using Clinitest® (Clinitest® Reagent Tablets, Bayer Corporation, Diagnostics Division, Elkhart, IN, USA), a common screening test used in the work up of metabolic and hepatic diseases. In the western population classical galactosemia is often suspected when non glucose reducing substances are detected in the urine of infants with cholestasis. However in East-Asian races the prevalence of classical galactosemia is very low whilst galactosemia due to altered uridine diphosphate-galactose epimerase activity in NICCD is more common. We present a case of NICCD in an East-Asian infant with cholestasis and persistently positive urine reducing substance.

Conclusion: NICCD deficiency should be considered as a differential diagnosis in any infant with cholestasis and persistently positive urinary reducing substances.

Keywords

Citrin deficiency, intrahepatic cholestasis, NICCD, urine reducing substance, galactosemia, fatty liver.
Corresponding author

Dr. Ajmal Kader (MBBS, MD, FRCPCH), KK Women’s and Children’s Hospital, Department of Pediatric Medicine, 100 Bukit Timah Road, Singapore 229899; telephone: +65 6394 2389; e-mail: ajmal.kader@kkh.com.sg.

How to cite

Introduction

Citrin deficiency (CD) or citrullinemia type 2 is an autosomal recessive metabolic disorder caused by mutations in SLC25A13 gene located on chromosome 7q21.3 [1]. A mutation in this gene has high prevalence in East-Asian races [2, 3] and leads to three distinct age dependent clinical phenotypes. It can manifest in newborns as neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) [4], in older children as failure to thrive and dyslipidemia caused by citrin deficiency (FTTDCD), and in adults/adolescents as recurrent hyperammonemia with neuropsychiatric symptoms in citrullinemia type II (CTLN2) [3, 5].

A range of metabolic derangements including galactosemia, citrullinemia along with increased levels of several other amino acids have been noted in NICCD [1, 3]. Urine for reducing substance (URS) is a non-specific screening test used to detect substances that chemically react with cupric sulphate [6]. A positive result is seen with common reducing sugars like glucose and galactose [6].

In classical galactosemia, the galactosemia is secondary to reduced Galactose-1-phosphate uridyl transferase (GALT) activity and the diagnosis is often suspected when URS is positive in infants with cholestasis. Patients with NICCD also present with cholestasis and positive URS, however the galactosuria is due to altered uridine diphosphate-galactose epimerase activity [1, 7]. With increasing global migration NICCD has now been reported from several western countries including a few in non East-Asian race [3]. We present an infant with normal newborn metabolic screening test who developed infantile cholestasis and had persistently positive URS which led to the diagnosis of NICCD.

Case report

A two month old female infant presented with one month history of worsening jaundice. She was born to non-consanguineous Chinese parents. A detailed three generation family history, antenatal and birth histories were unremarkable. She was predominantly breast fed and complemented with normal formula. She had neonatal jaundice which resolved spontaneously within 10 days. Postnatal metabolic screening on blood spot by tandem mass spectroscopy done at 48 hours of life showed normal thyroid functions, plasma amino acids and acyl carnitines.

Apart from jaundice, she was well and showed normal growth. Clinically infant was icteric, not dysmorphic and no hepatosplenomegaly was present. The stools were pigmented and urine was brownish-yellow. Preliminary investigations showed cholestatic jaundice with total bilirubin 171 (3-24 umol/L) and direct fraction 94 (2-7 umol/L). Serum gamma glutamyl transferase and aspartate aminotransferase were also raised. Fasting ultrasound abdomen showed fatty liver and a small gallbladder. Radio nucleotide scan showed good excretion of tracer into the small intestines at 4 hours effectively ruling out biliary atresia.

Investigations for cholestatic liver disease showed positive URS, but negative glucose on dipstick. The rest of the investigations including GALT activity and intrauterine/perinatal infection screen were not abnormal. She was commenced on ursodeoxycholic acid and multivitamins. Two weeks later her liver function test (LFT) showed improvement and URS remained positive. As she was clinically improving and classical galactosemia was ruled out, the URS was presumed to be due to transient galactosemia.

LFT done a month later showed a worsening trend (Tab. 1) and URS remained positive. Hence metabolic investigations were performed. Urine amino acids profile showed raised levels of several amino acids and specifically high citrulline levels at 1,033 (normal 0-10 umol/mmol). The serum amino acid profile showed markedly raised citrulline, threonine, methionine, lysine and arginine. The serum threonine: serine ratio was 2.6 (control 1.1). These findings are consistent with the diagnosis of NICCD. Serum galactose measured with fluorometer technique was raised indicating that the reducing substance in the urine was likely to be galactose. Her serum ammonia, lipid profile and lactate were normal.
Genetic testing confirmed the diagnosis of NICCD, it detected two heterozygous mutations, c.615+5G>A and c.851_854delGTAT (p.M285PfsX1) on SLC25A13 gene. Both mutations have been reported in patients with citrin deficiency ([2] and [8], respectively). She was commenced on lactose-free MCT enriched formula and 2 ½ months later the LFT and URS normalised.

### Discussion

SLC25A13 gene encodes for citrin, a mitochondrial aspartate-glutamate carrier protein, which is important in various metabolic pathways [1]. Mutations typically prevent cells from making any functional citrin, which causes derangements in aerobic glycolysis, gluconeogenesis, urea cycle, uridine diphosphate-galactose epimerase activity and fatty acid metabolism [1]. NICCD is associated with early onset cholestasis and growth retardation [3, 7]. Investigations may show intrahepatic cholestasis, hypoproteinemia, hypoglycemia, hyperammonemia, galactosemia, dyslipidemia and uridine diphosphate-galactose epimerase activity and fatty acid metabolism [1]. NICCD is associated with early onset cholestasis and growth retardation [3, 7]. Investigations may show intrahepatic cholestasis, hypoproteinemia, hypoglycemia, hyperammonemia, galactosemia, dyslipidemia and aminoacidemia including citrulline, methionine, threonine, and tyrosine [1, 3, 7]. NICCD is generally not severe and symptoms as well as biochemical findings often resolve by age one year with appropriate treatment. However, later on some patients may develop chubby facies, fatty liver, hyperammonemic neurological disturbances and liver failure [3, 7].

Over the last two decades Kobayashi and colleagues have extensively published on various aspects of CD and NICCD phenotype. In the largest case series on 75 cases of NICCD, they reported 40% detection rate on newborn blood spots screening, 70% of the patients had galactosemia along with raised methionine and/or phenylalanine [7]. In our country galactose assay is not included in the newborn screening, which may explain why our case was not detected earlier. Ohura et al. made a few important observations which may explain why some infants are not detected by screening test. They noted that the screen-negative patients did not have hypergalactosaemia at newborn screening, but developed it subsequently. They also observed that all the screen-positive patients manifested hypercitrullinaemia, whereas the amino acid concentrations in screen-negative patients were normal. Hence, they speculate that the screen-negative patients most likely developed the condition at a later time (infantile onset) [7].

URS (Clinitest® Reagent Tablets, Bayer Corporation, Diagnostics Division, Elkhart, IN, USA) is a simple non-invasive screening test often used in the work-up of intrahepatic cholestasis and metabolic disorders. Usual reducing substances examined are glucose and galactose. However, fructose, pentose, homogentisic acid and drugs like salicylates, levodopa, ascorbic acid, nalidixic acid, tetracyclines and probenecid are also associated with positive reaction [6]. Cephalosporins and hydrogen peroxide can give false negative results [6]. As our patient was not on medications the positive result is indicative of an underlying metabolic problem including galactosemia or proximal renal tubular dysfunction. In such clinical scenarios a positive URS and negative glucose on urine dipstick (diastase) often indicates galactosuria.

Classical galactosemia typically presents as infantile cholestasis and may lead to liver failure, often the diagnosis is suspected when galactose is detected by URS testing and confirmed by absent GALT activity in red blood cells. NICCD also presents in a similar manner but rarely leads to liver failure in infancy; the galactosuria in NICCD is due to altered uridine diphosphate-galactose epimerase activity and GALT activity is normal. The exact incidence of galactosemia and galactosuria in NICCD

### Table 1. Liver function test trend over 4 month period.

<table>
<thead>
<tr>
<th>Age (in days)</th>
<th>65</th>
<th>82</th>
<th>110</th>
<th>125</th>
<th>152</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate aminotransferases (16-56 U/L)</td>
<td>109</td>
<td>93</td>
<td>86</td>
<td>72</td>
<td>56</td>
<td>72</td>
</tr>
<tr>
<td>Alkaline phosphatase (157-363 U/L)</td>
<td>514</td>
<td>408</td>
<td>427</td>
<td>387</td>
<td>127</td>
<td>387</td>
</tr>
<tr>
<td>Gamma glutamyl transferase (3-24 U/L)</td>
<td>117</td>
<td>113</td>
<td>96</td>
<td>106</td>
<td>34</td>
<td>106</td>
</tr>
<tr>
<td>Total bilirubin (3-24 umol/L)</td>
<td>171</td>
<td>96</td>
<td>114</td>
<td>55</td>
<td>9</td>
<td>55</td>
</tr>
<tr>
<td>Direct bilirubin (2-7 umol/L)</td>
<td>94</td>
<td>55</td>
<td>67</td>
<td>38</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Albumin (38-53 g/L)</td>
<td>29</td>
<td>29</td>
<td>30</td>
<td>35</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td>Urine reducing substances</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>Not done</td>
<td>Negative</td>
<td>Not done</td>
</tr>
</tbody>
</table>

A case of NICCD in an East-Asian infant
patients is unknown. Ohura et al. in their study report galactosemia in 20 out of 30 patients [7].

In our infant, the URS was persistently positive, initially we were falsely reassured by the normal GALT activity and improving LFT. Investigations for non classical galactosemia due to glycerokinase or epimerase deficiency are recommended in symptomatic cases. URS can also be positive due to transient galactosemia and is often seen in infants who are otherwise well. Transient galactosemia is believed to be due to due to hepatic immaturity or due to abnormalities in hepatic vascular system [6, 9]. Metabolic investigations particularly plasma amino acid profile would aid in diagnosing NICCD. Genetic testing is essential to confirm NICCD [1, 3].

The prevalence of SLC25A13 mutation is more common in East-Asian Mongolian races. Carrier frequency has been reported as 1:112 in Koreans, 1:69 in Japanese and 1:48 in Southern Chinese [1-3]. Only 9 cases of CD type 2 have been reported in non East-Asian children [3]. Conversely, incidence of classical galactosemia is more common in Caucasians and rare in East-Asian Mongolian races [10, 11]. Given the relatively high incidence of SLC25A13 mutations in East-Asian population Naito et al. concludes that NICCD should be suspected in all cases of neonatal cholestasis with hypergalactosemia of unknown cause [12]. Therefore, an East-Asian infant with cholestasis and positive URS is more likely to have NICCD than classical galactosemia. With global migration of East-Asian races, NICCD is likely to be encountered by clinicians in western countries too. The few cases reported in other populations, may suggest it is a panethnic disease with a worldwide distribution.

Tazawa et al. describes liver histology findings of adult type fatty liver disease without evidence of giant cell hepatitis, in 3 infants with NICCD [3, 13]. Our case had sonographic evidence of fatty liver and small gall bladder at 2 months of age; we believe fatty liver at such an early age is a valuable clue and should alert the managing clinician about the possibility of NICCD.

Early management of NICCD is directed toward treating the consequences of cholestasis and galactosemia. Lactose free formula enriched with medium chain triglycerides (MCT) and supplementation with fat-soluble vitamins has been used to prevent complications in NICCD [3, 7]. How long this diet needs to be continued is not clear. Naito et al. performed lactose challenge tests on an infant with NICCD, the first challenge at 56 days led to worsening of LFT and hypergalactosaemia, whereas the re-challenge at 152 days did not worsen the laboratory findings. These data suggest that lactose may be toxic to patients with NICCD while cholestasis persists [12]. Our case improved on a MCT enriched lactose free formula and within three month the LFT returned to normal. A small percentage of infants with NICCD will develop CTLN2, hence ongoing health surveillance into adulthood is essential.

Conclusions

Presence of urine reducing substances in infants with cholestasis and/or fatty liver should alert clinicians about the diagnosis of NICCD. Normal newborn metabolic screening dose not exclude the diagnosis of NICCD. Metabolic screening with plasma amino acids and confirmation with mutation analysis for SLC25A13 gene is recommended. Unlike in western races, classical galactosemia is less likely to be the cause of hypergalactosemia in East-Asian population. With global migration of East-Asian races and a few reported cases in other races there is a need for awareness on NICCD and its surveillance into adulthood.

Declaration of interest

The Authors declare that there is no conflict of interest.

References


