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Title: Renal insufficiency mimicking glutaric acidemia type 1 in newborn screening

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Abstract

Background

Glutaryl carnitine (C5DC) in dried blood spots is used as a biomarker for glutaric aciduria type 1 (GA-1) screening. Since C5DC is the only screening marker for this condition, various pathological conditions may interfere with C5DC metabolism. Recently, it has been reported that cases of renal insufficiency showed C5DC elevation.

Method

Five cases who were positive for GA-1 via newborn screening using tandem mass spectrometry from September 2012 to March 2015 in our institute were enrolled in this study.

Results

GA-1 was not confirmed in any of the cases, using urinary organic acids analysis. C5DC level decreased immediately in 4 cases; however, one patient, who exhibited a high level of C5DC for at least 4 months, was diagnosed with bilateral renal hypoplasia.

Conclusion

In a case of persistently elevated C5DC, renal insufficiency should be considered as a differential diagnosis.

Key words

Glutaric acidemia I, glutarylcarnitine, renal insufficiency, neonatal screening.

Main text

Glutaric acidemia type 1 (GA-1) is a rare acidemia caused by an inherited deficiency of glutaryl-CoA dehydrogenase (GCDH) that could lead to severe motor disorder, cognitive impairment, and enlargement of the ventricle. Early diagnosis using newborn screening and the early implementation of medical and dietary treatment could prevent significant morbidity, mortality, and mental maldevelopment.[1] Glutaryl carnitine (C5DC) is an acylcarnitine derived from glutaryl-CoA and serves as the diagnostic biomarker used in tandem mass spectrometry-based newborn screening of GA-1 in several countries, including Japan.[2] GCDH defect gives rise to 3-hydroxyglutaric acid

(3OHGA) and glutaric acid (GA) in the urine and C5DC in the dried blood spot or plasma of a newborn. Recently, elevation of C5DC has also been reported in a case of other inherited metabolic disease, such as medium-chain acyl-CoA dehydrogenase deficiency.[3] It has also been observed that infants with congenital or acquired renal diseases showed a high level of C5DC [4]. In our institute, 5 cases of elevated C5DC level mimicking GA-1 were found via newborn screening. Four of the 5 cases showed a non-specific transient elevation of C5DC. However, one case of renal insufficiency retained a high level of C5DC for at least 4 months. Here, we describe the clinical characteristics of cases of elevated C5DC in newborn screening.

Cases

From September 2012 to March 2015, 5 cases were found positive for GA-1 with an elevation of C5DC in newborn screening and were referred to the metabolic outpatient clinic in Kobe University Hospital. Simultaneous elevation of other acylcarnitines was not noted. None of the cases exhibited neurological signs that would indicate GA-1, such as macrocephaly, dystonia, and dyskinesia. In all cases, urinary organic acids analysis using gas chromatography-mass spectrometry was performed after the first visit to our hospital. None of the cases demonstrated an excess excretion of GA and 3OHGA in the urine. Moreover, GA-1 was not confirmed in all cases. Repeat examination demonstrated that the C5DC level decreased within a month after initial elevation in 4 cases. However,

a high C5DC level persisted for at least 4 months in one case (Table 1). The patient in this case was the second twin delivered by Cesarean section at 36 gestational weeks. His birth weight was 1850 g, which was smaller than that of his brother, who weighed 2340 g.

His C5DC level in dried blood spot at the age of 1 month was 0.25 nmol/mL (cut-off value: 0.25nmol/mL). At the first visit, he showed failure to thrive. Careful, in-depth evaluation revealed elevated serum creatinine, low estimated glomerular filtration rate (24 mL/min/1.73 m^2 calculated using the original Schwartz method [5]), metabolic acidosis with normal anion gap, and renal tubular damage with increased N-acetyl-beta-D-glucosaminidase (6.1 U/L, reference value: 0 to 5.7) and beta 2-microglobulin (26561 μg/L, reference value: 0-289), indicating renal insufficiency. Diagnostic abdominal ultrasonography ultimately revealed bilateral renal hypoplasia. The longitudinal dimensions of the right and left kidney were 39.0 mm and 39.7 mm, respectively, and were smaller than the reference dimensions of the kidney of an infant aged 1 to 3 months (mean ± SD: 50.0 ± 5.5 mm, 5 to 95th percentile: 42 to 59 mm)[6] (Figure 1). At the age of 4 months, his C5DC level remained high (0.29 nmol/mL, cut-off value: 0.25). His serum albumin, blood urea nitrogen, and hematocrit levels were 4.5 g/dL (reference value: 4.1-5.0), 25.2 mg/dL(9.0-22.0), and 36.1% (39.0-52.0%), respectively, thereby indicating dehydration due to renal insufficiency. This results in slightly higher C5DC levels. At the age of 6 months, special milk of 8806H formula (Na;

2.7 mEq/100 mL, K 0.8 mEq/100 mL and P 24 mg/100 mL) was administered for renal insufficiency. His renal dysfunction persisted until at least the age of 2 years and 6 months, at which time the patient exhibited a low estimated glomerular filtration rate that ranged from 39.4 to 45.1 mL/min/1.73 m^2.

Discussion

In our cases of elevated C5DC level, 4 cases showed transient elevation. Conversely, a case of renal hypoplasia retained a high C5DC level. Elevated C5DC in newborn screening is considered a GA-1 case.[2] However, none of our 5 cases of elevated C5DC level had GA-1. Since C5DC is the only variable used for GA-1 screening, an unacceptably high rate of false positives and the risk of less-than-100% sensitivity are concerning. It is also difficult to distinguish true GA-1 from other diseases, using only C5DC quantification, since patients with GA-1 do not always show elevated C5DC levels.[7] Additionally, some patients may demonstrate either no, intermittently increased, or borderline elevated C5DC concentrations.[8] Moreover, analyses of C5DC may show a considerable inter-assay variability.[2] In fact, our 4 cases were confirmed as false positive cases by urinary biochemical analysis. C5DC level in the false positive cases decreased over a short time, thereby indicating that repeat examination following initial elevation is necessary to avoid a risk of less diagnostic sensitivity. One case had a persistently high level of C5DC for at least 4 months. This case involved bilateral renal hypoplasia

and showed impaired renal function. Three cases of transient elevation of C5DC did not show elevated creatinine (Table 1). Recently, it has been reported that neonates with renal failure showed an elevation of C5DC and mimicked GA-1 findings in the newborn screening.[4] In the case of renal failure, concentration of C5DC in the dried blood spot correlated with the level of serum creatinine and glomerular filtration rate.[4,9] This suggests that a C5DC positive case may be considered not only to have GA-1, but also to have renal insufficiency, in order to administer appropriate treatment.

The mechanism that led to elevation of blood C5DC level in the case of renal insufficiency is unclear. Recently, sodium-dependent dicarboxylate cotransporter 3 (NaC3) and organic anion transporter (OAT) 1 and 4 have been identified to mediate the translocation of GA and 3OHGA through the membrane.[10] In *Gcdh*-/- mice (a mouse model of GA-1), NaC3 and OAT1 expression was increased in the kidneys, indicating an adaptive response to increased plasma GA and 3OHGA levels. When metabolic crisis was induced in the Gcdh-/- mice, OAT1 was mislocalized in the tubule cells, and histomorphological changes in the kidneys, contributing to functional tubular injury, were induced.[10] These findings suggest that renal tubular cells play an important role in the translocation of metabolites GA and 3OHGA in the GA-1 mouse model. Therefore, renal insufficiency involving the tubular cells may alter the excretion level of metabolites and result in accumulation of GA, 3OHGA, and C5DC.

To ensure high diagnostic sensitivity in newborn screening for GA-1, a combination of C5DC with secondary variables, such as C5DC/(C8+C10), C5DC/C16, C5DC/C0, and C5DC/C8, has been proposed.[2,4] However, since not only our local laboratory but also other local laboratories in Japan measure single values of C0, C8, C10, and C16, established cut-off values for C5DC/acylcarnitine ratios are not available. Therefore, in Japan, the acylcarnitine profile alone is insufficient for distinguishing renal insufficiency from GA-1. Urinary organic acid analysis is essential for confirming diagnosis; however, this test requires additional time for completion and obtaining results. Serum creatinine, urinary N-acetyl-beta-D-glucosaminidase, and beta 2-microglobulin assays are widely available in laboratories and less time-consuming for estimating renal insufficiency. The renal function test should be considered in cases of C5DC elevation without abnormal neurological signs for differential diagnosis.

Disclosure statements

The authors declare no conflict of interest

Author contribution

M.M. and H.A. wrote the manuscript. H.A., M.Y., and T.K. collected and provided clinical data; R.B. and Y.H. analyzed the urine samples. M.N., K.T., M.N., H.N., and YT

critically reviewed the manuscript. I.M., Y.H., and K.I. gave conceptual advice. All authors read and accepted the final manuscript.

References

1. Bijarnia S, Wiley V, Carpenter K, Christodoulou J, Ellaway CJ, Wilcken B. Glutaric aciduria type I: outcome following detection by newborn screening. *J. Inherit. Metab. Dis*. 2008; 31(4): 503-7.

2. Lindner M, Ho S, Fang-Hoffmann J, Hoffmann GF, Kolker S. Neonatal screening for glutaric aciduria type I: strategies to proceed. *J. Inherit. Metab. Dis*. 2006; 29(2-3): 378-82.

3. Napolitano N, Wiley V, Pitt JJ. Pseudo-glutarylcarnitinaemia in medium-chain acyl-CoA dehydrogenase deficiency detected by tandem mass spectrometry newborn screening. *J. Inherit. Metab. Dis*. 2004; 27(4): 465-71.

4. Hennermann JB, Roloff S, Gellermann J, Gruters A, Klein J. False-positive newborn screening mimicking glutaric aciduria type I in infants with renal insufficiency. *J. Inherit. Metab. Dis*. 2009; 32 Suppl 1: S355-9.

5. Schwartz GJ, Feld LG, Langford DJ. A simple estimate of glomerular filtration rate in full-term infants during the first year of life. *J. Pediatr*. 1984; 104(6): 849-54.

6. Konus OL, Ozdemir A, Akkaya A, Erbas G, Celik H, Isik S. Normal liver, spleen, and kidney dimensions in neonates, infants, and children: evaluation with sonography. *AJR Am. J. Roentgenol.* 1998; 171(6): 1693-8.

7. Viau K, Ernst SL, Vanzo RJ, Botto LD, Pasquali M, Longo N. Glutaric acidemia type 1: outcomes before and after expanded newborn screening. *Mol. Genet. Metab.* 2012; 106(4): 430-8.

8. Lindner M, Kolker S, Schulze A, Christensen E, Greenberg CR, Hoffmann GF. Neonatal screening for glutaryl-CoA dehydrogenase deficiency. *J. Inherit. Metab. Dis*. 2004; 27(6): 851-9.2

9. Goek ON, Doring A, Gieger C, Heier M, Koenig W, Prehn C, et al. Serum metabolite concentrations and decreased GFR in the general population. *Am. J. Kidney. Dis.* 2012; 60(2): 197-206.

10. Thies B, Meyer-Schwesinger C, Lamp J, Schweizer M, Koeller DM, Ullrich K, et al. Acute renal proximal tubule alterations during induced metabolic crises in a mouse model of glutaric aciduria type 1. *Biochim. Biophys. Acta*. 2013; 1832(10): 1463-72.

Table 1. Cases of elevated C5DC in newborn mass-screening

Case	Initial C5DC level in dried blood spot (nmol/mL)	Cut-off value (nmol/mL)	Duration of elevation	Serum creatinine level(mg/dl)	Diagnosis
1	0.35	0.28	1 month	0.28	False positive
2	0.40	0.30	1 month	0.17	False positive
3	0.39	0.30	1 month	Not examined	False positive
4	0.35	0.30	1 month	0.24	False positive
5	0.29	0.25	>4 months	0.81	Renal hypoplasia

Figure 1