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Neonatal ketosis is not rare: experience of neonatal screening using gas chromatography–mass spectrometry

Takahiro Inokuchi[a,*], Ichiro Yoshida[a,b,c], Akiyo Kaneko[a], Kyoko Tashiro[a], Satomi Tashiro[a], Misa Jogo[a], Kumiko Aoki[a], Masatoshi Tanaka[a,d]

[a]*Research Institute of Medical Mass Spectrometry, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*

[b]*Department of Pediatrics, Kurume University School of Medicine, Kurume, Japan*

[c]*Department of Medical Education, Kurume University School of Medicine, Kurume, Japan*

[d]*Department of Pharmacology, Kurume University School of Medicine, Kurume, Japan*

Abstract

The causes and effects of transient neonatal ketosis, discovered during a pilot study of screening for abnormalities in neonatal metabolism using gas chromatography–mass spectrometry, were investigated. Of the 21 342 neonates that were screened, 47 had significant ketosis. The organic acid profile accompanying ketosis in the urine of neonates followed the pattern of ketotic dicarboxylic aciduria in approximately half of the cases. Ketosis was more often found in neonates nourished by breast feeding (33 out of 47). Over half of the neonates showing ketosis (28 out of 47) were asymptomatic. When normal neonates and neonates testing positive for ketosis were compared, no statistically significant correlations were found with regard to birth mass, gestational period, or gender. However, neonates with ketosis tended to have low mass gain rates in the 5 days from birth and a statistically significant difference was found in this regard in comparison to normal neonates ($P<0.0001$). From the above results, development of ketosis in neonates was found to be possible even in normal subjects. Most ketosis in neonates was also found to depend largely on nourishment after birth. Existence of an asymptomatic ketosis category was also suggested.

Keywords: Neonatal ketosis

1. Introduction

In the course of researching a screening test for neonatal inherited metabolic disorders using gas chromatography–mass spectrometry (GC–MS), the authors tested the urine of 21 342 neonates between January 1996 and August 2000. Urinalysis revealed various metabolic abnormalities, including one case of citrullinemia, two cases of glycerol kinase deficiency, one case of methylmalonic aciduria, one case of ornithine transcarbamylase deficiency, two cases of 2-ketoadipic aciduria, two cases of neuroblastoma and 19 cases of transient neonatal tyrosinemia. In this way the study contributed to the early detection of these metabolic abnormalities and the prevention of damaging symptoms. At the same time, since GC–MS is used as the analysis method for the screening, marker materials for discovery of metabolic abnormalities as well as 3-hydroxybutyric acid, used as the ketosis index, and other metabolic

*Corresponding author. Tel.: +81-942-317-582; fax: +81-942-313-961.

E-mail address: takain55@med.kurume-u.ac.jp (T. Inokuchi).

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products in urine can be simultaneously analyzed accurately and with high sensitivity. Through this pilot screening study for metabolic abnormalities using GC–MS, the possibility of ketosis in neonates thought to be normal was demonstrated. For the 47 normal neonates showing significant ketosis, factors thought to be closely related to the condition were investigated. Specifically, birth mass, mass increase rates, gestational period, nourishment after birth, and other factors were investigated to determine any possible links to neonatal ketosis.

2. Experimental

2.1. Test subjects

Of the 21 342 subjects screened between January 1996 and September 2000, all 47 neonates showing significant ketosis were selected. The relationship between neonatal ketosis and various factors, including birth mass, gestational period, gender, nourishment, presence of sickness, and rate of mass gain was then investigated.

2.2. Analysis method

After getting parental informed consent, urine samples were obtained from neonates between 4 and 6 days old and then analyzed. Organic acid in the urine was extracted according to the methods reported by Shoemaker and Elliot [1] and Matsumoto and Kuhara [2]. A 100-nmol concentration of deuterium-labeled creatinine (d_3-creatinine) and 50 nmol of heptadecanoic acid (HDA) were added as internal standards for quantitative analysis of creatinine and organic acid. The extracted urine was dried under nitrogen gas flow and the TMS derivative was made. A total of 1 μl of this solution was injected into the GC–MS system and analyzed [3]. Neonates with urine containing 3-hydroxybutyric acid (3HB) in quantities exceeding 10 times the normal level [mean+3SD=54.9 mmol/mol creatinine (Cr)] were considered to have significant ketosis and enrolled as subjects of this study. When testing a statistical hypothesis among the various factors and groups with or without ketosis, an unpaired Student's *t*-test and Welch's *t*-test were used for analyzing parametric variables and a Mann–Whitney *U*-test for analyzing non-parametric variables. A chi square test and Fisher's exact test were used to compare categorical variables. All statistical tests were two-tailed and significance was defined as $P<0.01$. Also, in the present study, statistical analyses were performed using statistical software, StatView 5.0 (SAS Institute).

3. Results and discussion

A typical total ion current (TIC) chromatogram obtained from the urine of a neonate with ketosis using the GC–MS analysis screening method is shown in Fig. 1. Given that the retention time for the 3HB peak, the marker material for ketosis, is the same as the retention time for the peak of 3-hydroxyisobutyric acid (3HiB) when using this analysis method, it is important to distinguish between these two chemicals [4]. The mass spectra of 3HB and 3HiB are shown in Fig. 2. Although the molecular ion for both chemicals is *m/z* 248, 3HiB has a mass spectrum ion at *m/z* 177 while 3HB has an ion at *m/z* 191. These ions are very important in distinguishing between the two chemicals. As a result, if the *m/z* 177 and *m/z* 191 ions are used for quantitation, both chemicals can be accurately and separately quantified.

Conventionally, normal infants immediately after birth rarely develop ketosis. One cause of this is known to be mitochondrial hydroxymethylglutaryl-

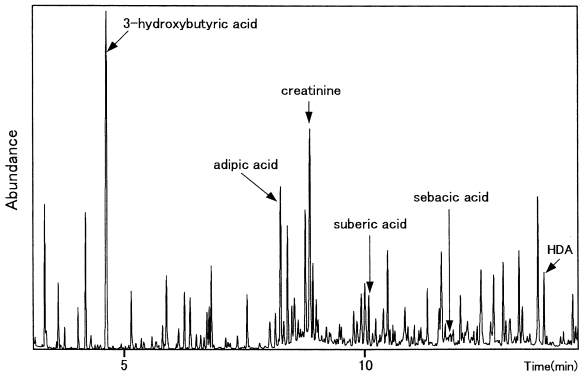

Fig. 1. Typical TIC chromatogram of TMS-derivatives of urinary metabolites from a newborn with ketosis.

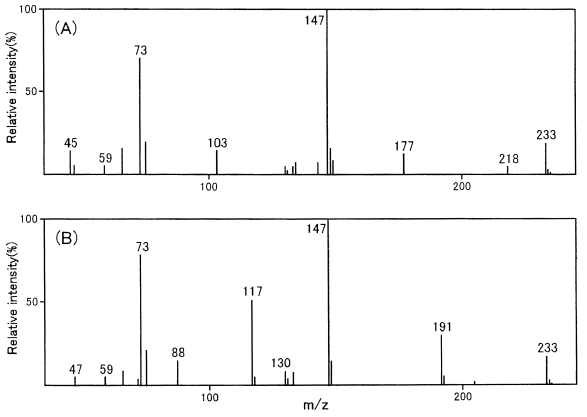

Fig. 2. Mass spectra of TMS-derivatives of 3-hydroxyisobutyric acid (A) and 3-hydroxybutyric acid (B).

CoA (HMG-CoA) synthase activity, which is lower than the activity of other ketone producing enzymes during neonatal period [5]. When neonates show ketosis, conventional texts have suggested that these neonates must have propionic acidemia or methylmalonic acidemia. Of the 21 342 subjects screened in this study, we found 47 cases of significant ketosis, in which 3-hydroxybutyric acid was detected in the urine at more than 10 times the normal level. Of these cases, four received an insufficient amount of mother's milk, two showed some jaundice, two had vomiting, and 11 had clearly poor mass gain. A total of 19 therefore showed some symptoms while the other 28 showed no symptoms at all. In other words, since over half of all neonates with ketosis showed no symptoms, the presence of an asymptomatic ketosis category in neonates was demonstrated. In addition, 28 of the 47 ketosis cases resulted from increases in dicarboxylic acids, including adipic acid, suberic acid, and sebacic acid. An elevated level of adipic acid was found in 26 cases, elevated suberic acid in 19 cases, and elevated sebacic acid in 11 cases. When nourishment in the ketosis cases was investigated (Table 1), a large number, 33, were found to be receiving mother's milk (70.2%). These results suggest that ketone increases are caused by accelerated fatty acid decomposition, due to insufficient supply or use of sugars, ketone production based on low acetyl-CoA processing capability in the tricarboxylic acid (TCA) cycle, and accelerated β oxidation of fatty acids.

Table 2 shows the results of a statistical analysis of each factor relative to the presence or absence of ketosis in neonates. No statistical evidence was found to have relationship with birth mass, gestational period, and gender with the presence or absence of ketosis. However, there was a nearly twofold difference in the rate of mass gain in the 5 days after birth when neonates with ketosis (mean$=-0.085$, $n=18$) were compared to neonates without ketosis (mean$=-0.042$, $n=110$). Since a normal distribution could not be confirmed for mass gain rates in the ketosis group ($n=18$), the Student's *t*-test, the Welch's *t*-test, and the Mann–Whitney *U*-test were used. The results of the three tests were the same, with showing a significant difference in mass gain rates between the two groups ($P<0.0001$). A chi square test with Yates' correction and Fisher's exact test were used to analyze differences in ketosis positive rates between mother's milk (0.47%: 33/6974) and combination milk (0.11%: 14/13 122) groups utilizing a 2×2 cross table. The results were the same for the two tests ($P<0.0001$), thus clarifying that neonate ketosis is more common among mother's milk-fed infants. In summary, the mass loss rate for ketosis cases in the 4 days after birth was large and the infants were often nourished by mother's milk. In other words, the newborn ketosis cases found in the screening process using GC–MS were often asymptomatic and nourishment had a major impact.

From the above results, the possibility for ketosis

Table 1
Nourishment to newborn

Nourishment	Ketosis (+)	Ketosis (−)
Mother's milk	33	6941
Formula	0	1013
Combination (mother's milk+formula)	14	13 108
Total	47	21 062

Table 2
Statistical examination of relationship between various factors and ketosis

Factor	Ketosis (+)	Ketosis (−)	*P* value
Male/female	24/23	10 952/10 293	0.5848[a]
Mother's milk/combination	33/14	6941/13 108	<0.0001[a]
Gestational period (mean±SD)	39.5±1.2	39.3±1.1	0.1930[b]
	(*n*=45)	(*n*=20 266)	0.1533[c]
Birth mass (mean±SD)	3197.0±359.0	3110.5±336.3	0.0815[b]
	(*n*=46)	(*n*=19 575)	0.0969[c]
Mass gain rate (mean±SD)	−0.085±0.022	−0.042±0.023	<0.0001[d]
(5 days after birth)	(*n*=18)	(*n*=110)	<0.0001[c]

[a] Fisher's exact test.
[b] Unpaired Student's *t*-test.
[c] Mann–Whitney's *U*-test.
[d] Welch's *t*-test.

to develop in normal neonates was demonstrated. As with asymptomatic hypoglycemia, existence of an asymptomatic ketosis category was suggested in the newborn period. However, ketones often have special roles, in such organs as the brain and lungs, during the newborn period [6,7] and future investigations should focus on the effects of asymptomatic ketosis in development processes after this period.

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