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Letter to the Editor

Diagnosis of organic acidemia in developing countries

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Dear Editor,

The analysis of organic acids in human urine by gas chromatography–mass spectrometry (GCMS) has become routine in the United States, Europe and Japan. By this technique it is possible to diagnose many inborn errors of amino acid, lipid and carbohydrate metabolism [1–4]. Some developing countries, however, still have limited access to GCMS. We report here the diagnosis of several cases of organic acidemia by GCMS of urine collected from patients in Brazil and Chile, absorbed on filter paper, and mailed to the United States or Japan for analysis.

Patient urine obtained in Brazil and Chile were analyzed locally for creatinine. A volume of urine equivalent to 0.5 mg creatinine (1–2 ml) was applied to a filter paper together with 20 μg of heptadecanoic acid as an internal standard. Filter paper of medium porosity, such as a 12 cm Whatman No. 2 or a Guthrie screening filter paper would hold 1–2 ml of urine. The filter paper was air dried and mailed. The referral laboratory extracted the urine sample absorbed in the filter paper into 2 ml of H_2O. After acidifying to pH 2 with 1 N HCl and saturation with NaCl, this extract was extracted twice with 2 ml of ethyl acetate. The combined organic layers were dried with anhydrous sodium sulfate, and evaporated to dryness under nitrogen. One hundred μl of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylcholorosilane (Pierce Chem. Co., Rockford, IL) was added, and the sample was trimethylsilylated at 60 °C for 30 min. GCMS analysis was carried out with a Finnigan 3300 mass spectrometer at Institute for Basic Research, New York, and with a JEOL DX303 Mass Spectrometer at Kanazawa Medical University, Japan. Mass spectra were obtained in the EI mode and characterized by comparing them with the library spectra of standard organic acids [4].

Serum phytanic acid was analyzed by the direct transesterification and capillary gas chromatographic method [5], except that the identification of methyl phytanate was made by Finnigan 3300 mass spectrometer operated in the chemical ionization mode using methane as the reagent gas, and the M + H ion (m/z 327) of the methyl phytanic acid was monitored and quantitated.

The results of the analysis of urine from eight patients, and serum from one patient are summarized in Table I. From the excessive excretion of urinary glutaric

TABLE I

Urinary (serum) organic acids (Analyzed in the US and Japanese laboratories)

Patients	Organic acids	μg/mg Creatinine *		Diagnosis	Case history
		USA	Japan		
1	GA	86	6	Glutaric aciduria I	A girl from Polish and German parents, was born after a normal pregnancy. At 10 mth, she had gastroenterocolitis with focal and generalized seizures lasting a few minutes. From that time she lost head control, and her motor development was poor. Neurological examinations showed head circumference of 48 cm, spastic diplegia, dystonic movements in arms and head, and bilateral Babinski response. The CT scan showed diffuse cortical atrophy. A skin fibroblast culture of the patient was prepared and the activity of glutaryl-CoA dehydrogenase in the culture was found to be only 2.1% of the control. The patient received a low protein diet and later a lysine-free diet, Lys I (Milupa Corporation). Some slow improvement was observed, but the patient died at age 4–5 yr following bronchopneumonia.
	3HGA	10	5		
2	GA	32	34	Glutaric aciduria I	3-mth-old boy European descent, died following a coma of unknown origin.
	3HGA	10	4		
3	GA	810	540	Glutaric aciduria I	13-mth-old boy of European descent, had a history of vomiting and of lethargy during viral disease. He developed hyperammonemia (300 mg/100 ml). After he had been placed on a low protein diet, vomiting and other symptoms disappeared. He has developed normally since.
	3HGA	32	20		
4 **	GA	2,000	2,644	Glutaric aciduria I	7-yr-old boy, had a healthy development during the first month of life. He had macrocephaly and big ventricles, and received a cerebro-peritoneal shunt. He developed
	3HGA	120	92		

					involuntary extrapyramidal movement and motor impairment. Since being on low tryptophan diet, his involuntary movements have improved. He has a normal IQ.
5**	GA	31	22	Glutaric aciduria I	6-yr-old boy, has developed normally and is on normal diet.
	3HGA	12	8		
6**	GA	980	932	Glutaric aciduria I	2-yr-old boy, had mild macrocephaly but developed normally until he was 13 mth old when he had seizures. He is mentally retarded with extrapyramidal movements. He is currently on low tryptophan diet, but the improvement is not significant.
	3HGA	55	40		
7**	GA	70	61	Glutaric aciduria I	9-mth-old girl, is completely normal, and is on a normal diet.
	3HGA	15	6		
8	3HPA	205	195	Propionic acidemia	4-mth-old boy of European descent. Three days after birth he developed vomiting, lethargy, seizures, coma and hyperammonemia. He was put on a low protein diet after severe metabolic derangement was suspected. He died at 6.5 mth when he had bronchopneumonia. Urinary organic acid indicated a propionic acidemia.
	MCA	420	379		
	LA	75	57		
9	Phytanic acid	20 (μg/ml serum)		Refsum disease	6 yr-old female of Portuguese descent, started to show symptoms of difficulty in seeing at age three when motor astigmatism was diagnosed. The opthalmologic examination showed retinitis pigmentosa. The patient has been placed on a diet low in phytanic acid, but there is no evidence of improvements in her condition.

Abbreviations: GA, glutaric acid; 3 HGA, 3-hydroxyglutaric acid; 3HPA, 3-hydroxypropionic acid; MCA, methyl citric acid; LA, lactic acid.

* 24-h urine from 10 healthy children 1–4 yr were used as the control value. The control urine contained GA 1.6–2.0 μg/ml creatinine, lactic acid 5.6–9.6 μg/mg creatinine; no 3HGA, 3HPA and MCA were detected in the controls.

** Case 4 to 7 are siblings in a family of European descent. Four out of five siblings in the family have high concentrations of urinary glutaric acid, but a 5-yr-old brother has normal urinary glutaric acid level and has developed normally. Case 6 is the index case of the family.

acid and 3-hydroxyglutaric acid, patients 1 to 7 were identified to suffer from glutaric aciduria Type I. Patient 8 was diagnosed as propionic acidemia from the large amount of urinary 3-hydroxypropionic and methylcitric acid. The serum phytanic acid level of patient 9 was elevated to 20 μg/ml, indicating Refsum's disease. The normal level of serum phytanic acid is < 2 μg/ml [6,7].

As shown by our findings, analysis of specimens collected on an ion-exchange filter paper [8,9] or Guthrie's screening filter paper [10] can overcome difficulties in shipping samples. We performed duplicate analyses at two sites to show that the samples were stable during the mailing and resulted in the same diagnosis. Recently, the International Chemical Diagnosis System for Inborn Errors of Metabolism has been established to exploit the described approach.

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