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Original Article

Role of Plasma Amino Acids and Urinary Organic Acids in Diagnosis of Mitochondrial Diseases in Children

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ABSTRACT

BACKGROUND: Diagnostic difficulty in mitochondrial diseases (MD) results not only from the wide spectrum of symptoms and signs but also from the absence of a reliable screening or diagnostic biomarker. **AIM:** To investigate the likelihood of MD in patients with symptoms and signs impressive of MD through quantitative measurement of plasma amino acids, and urinary organic acids. **METHODS:** Twenty patients with symptoms and signs suggestive of MD were further evaluated by quantitative plasma amino acids and urinary organic acids assay and neuroimaging. **RESULTS:** Plasma amino acid results revealed elevation of alanine in 11, glycine in five, and proline in two patients. Abnormal urinary organic acid analysis was present in six patients; increased urinary lactate (20%), dicarboxylicaciduria (15%), and urinary ketone bodies (10%). Upon enrollment our patients scored as possible MD according to the MD scoring system. At the end of the study, five patients still scored as possible MD, eight patients as probable MD, and seven patients as definite MD. All patients with definite MD had elevated serum lactate. In three patients, elevated urinary lactate was the only abnormality. Alanine was elevated in all patients with definite MD, whereas proline was elevated in only one. Magnetic resonance imaging of the brain showed atrophic changes in one patient and bilateral basal ganglia hyperintensity in another. **CONCLUSION:** Urinary organic acids and quantitative plasma amino acids can help in the diagnosis of MD, especially when the economic burden and absence of specialized centers limits the diagnosis.

Keywords: mitochondrial disease, plasma amino acids, urinary organic acids

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Introduction

Mitochondrial diseases (MD) are usually progressive multisystem disorders. Identifying suspected mitochondrial dysfunction is not a simple task. That is because no single biomarker identifies all, or even most, cases with sufficient sensitivity or specificity.[1] Over the past few decades, several sets of diagnostic criteria were developed to assist in the recognition and diagnosis of mitochondrial disorders.[2-5] All of these are based on some combination of clinical, laboratory, pathologic, biochemical, and genetic findings. This creates a huge economic burden in countries with limited resources where many of the investigations must be self-paid.

We investigated the likelihood of MD in patients with symptoms and signs that are suggestive of the disorder through simpler and more readily available tests; these studies included quantitative measurement of plasma amino acids and urinary organic acids, serum lactate and ammonia, and neuroimaging. We applied the 2002 Wolf/Smeitink criteria[5] for diagnosis in this group of patients.

Methodology

Over a 5-month period, we studied 20 consecutive patients who presented to the Pediatric Neurology Outpatient clinic at Ain Shams

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University Hospital with clinical symptoms and signs that classify them as possible MD on the Mitochondrial Disease Criteria (MDC) score.[5]

In addition to basic laboratory investigations (complete blood picture, kidney and liver function tests, fasting blood glucose, serum ammonia, serum lactate, and blood gas analysis), amino acids were quantitatively measured (using plasma amino acid analyzer), and urine organic acids were semiquantitatively assayed (using gas chromatography).

We stressed on amino acids associated with mitochondrial dysfunction, especially alanine. An absolute elevation in alanine above 450 μM/L is a factor used to determine the likelihood of mitochondrial disease in the Nijmegen diagnostic protocol.[5] Other amino acids whose elevation has been associated with mitochondrial dysfunction include proline, glycine, and sarcosine.[6]

Urine organic acids reported to be associated with mitochondrial diseases include the tricarboxylic acid cycle intermediates: ethylmalonic acid and 3 methylglutaconic acid,[7] lactic aciduria,[3] dicarboxylicaciduria: oxalic, malonic, succinic, glutaric, adipic, pimelic, suberic, azelaic and sebacic acids,[6] and ketones: B–hydroxybutyrate and acetoacetate.[6]

All patients underwent formal ophthalmological examination and neuroimaging: 10 patients underwent computed tomography, and 19 underwent magnetic resonance imaging (MRI) (one patient died).

Limitations of the study

The patients never underwent definitive testing and there was no control group to test sensitivity and specificity.

Statistical methodology

Statistical analysis was done using manual methods to calculate percentage, mean, and standard deviation of collected patients' data.

We rigidly applied the 2002 Wolf/Smeitink criteria in this group of patients and the following are our results.

Results

Clinical manifestations, laboratory test results, and cranial MRI findings of the included patients are summarized in Tables 1 and 2. They were 16 boys and four girls. Their age ranged between 7 months and 11 years, with a mean of 50 ± 38.7 months. The age of onset of disease ranged from as early as 3 days to 5.25 years with a mean of 1.00 ± 1.21 years.

Clinical evaluation of the studied patients showed that neurological manifestations were the most common presentations, the most common of which was mental retardation, seen in 19 patients (95%). Convulsions, delayed motor development, and speech abnormalities were each observed in 18 patients (90%) and muscle weakness in 16 patients (80%). Other manifestations included failure to thrive (60%), ophthalmic manifestations (35%), gastrointestinal manifestations (30%), and hepatomegaly (20%).

Basic laboratory investigations showed that 15 patients (75%) had elevated serum ammonia and 13 patients (65%) had elevated serum lactate, whereas liver transaminases were elevated threefold in two patients. Serum creatinine and fasting blood glucose were within normal in all included patients.

Plasma amino acid analysis results revealed elevated alanine in 11 patients (55%), whereas glycine was elevated in five (25%) and proline in two patients (10%). Abnormal plasma amino acids with raised alanine and/or proline with coexisting/concurrent lactic acidosis, was present in 10 patients (50%) (Table 2).

Urinary organic acid analysis was abnormal in six patients (30%); three patients with isolated increased lactate excretion, one patient with increased urinary lactate as well as ketones (B-hydroxybutyrate, and acetoacetate) and adipic acids, and one patient with increased urinary succinate. The latter patient had increased urinary acetoacetate and adipic acids levels.

MRI of the brain was done for 19 patients (one patient died). Neuroimaging revealed atrophic changes in six patients, basal ganglia abnormalities in three (one with changes consistent with Leigh syndrome, a second with basal ganglia hyper-intense signals on T_2, and a third with basal ganglia calcification). White matter abnormalities were detected in three patients (two with white matter abnormal high signals on T_2 and a third with extensive deep white matter high signals affecting the subcortical area) (Table 2).

Fundus examination was done in all patients except one who had bilateral congenital cataract. This revealed a pale optic disc with attenuated blood vessels in one patient only.

Upon enrollment, our 20 patients had scored 2-4 with possible MD based on their clinical and few basic laboratory findings according to mitochondrial disease scoring system.[5] At the end of the study, seven patients could be scored as definite and eight as probable MD, with only five patients who still scored as possible MD. Notably, those patients had abnormal elevation of other urinary organic acids and plasma amino acids than those listed in MDC by Wolf and Smeitink.[5] They are, however, known to be commonly elevated in MD.[3,6,7]

All patients considered as having MD who were enrolled in the study had elevated serum lactate, whereas six patients only had elevated serum ammonia. Plasma alanine was elevated in all patients whereas proline was elevated in only one. Urinary lactate was elevated in three patients.

Discussion

Genetically based, primary mitochondrial dysfunction represents a heterogeneous group of disorders that are now recognized to constitute the most common neurometabolic disorder of childhood.[8] Being a third-world developing country, we have very limited financial support. Therefore, we need to discuss the role of preliminary investigations as quantitative plasma amino acids and urine organic acids in confirming the diagnosis of mitochondrial disease in those suspected to have the disorder.

Using the 2002 Wolf/Smeitink criteria,[5] quantitative plasma amino acids and urine organic acids assays could help in identifying seven patients with MD from 20 suspected of having the disease. In those patients, the first presenting symptoms of the disease were observed as early as 3 days to 1 year of life. The onset of symptomatic MD is usually in the first decade of life;[9] our patients presented at a mean age younger than that reported by El Bassyouni et al. and Scaglia et al.[10,11] (3.3 years in a group of 400 children diagnosed as having an MD according to the modified Walker criteria). In our study, there was a male predominance (80% of the studied patients). This agrees with