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Clinical Value of Magnetic Resonance Spectroscopy in the Initial Evaluation of Patients with Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes

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Purpose: Magnetic resonance spectroscopy (MRS) is a diagnostic tool used to detect abnormal accumulation of lactate in the brain parenchyma in various metabolic diseases. This study evaluated the clinical roles of brain MRS in the initial assessment of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) caused by impairment of the mitochondrial respiratory chain.

Methods: Twenty-five patients with the A3243G mutation among 34 MELAS patients referred to the pediatric neurology clinic of Gangnam Severance Hospital between January 2006 and December 2020 were included. In this retrospective study, demographic, clinical, laboratory (serum lactate and lactate-to-pyruvate ratio), magnetic resonance imaging (MRI), and initial MRS (presence of lactate peak and abnormal N-acetylaspartate [NAA]) data were reviewed.

Results: Brain MRI showed cortical lesions in 24 of 25 genetically confirmed A3243G MELAS patients with neurologic symptoms in this study. On MRS, 18 patients (72%) had increased lactate peaks, depicting anaerobic energy metabolism, and 17 patients (68%) had decreased NAA levels, indicating neuronal integrity. Ten patients underwent MRS in the acute stage (within 2 weeks of symptoms). Unlike patients who underwent MRS more than 2 weeks after symptom onset, a lactate peak on MRS was observed in all patients in the acute stage (P=0.011).

Conclusion: Elevated lactate peaks in acute cerebral infarctions are highly suggestive of mitochondrial encephalopathy. MRS alone is insufficient to diagnose MELAS, but it is valuable as a noninvasive supplemental diagnostic tool in combination with genetic testing.

Keywords: Magnetic resonance spectroscopy; Mitochondrial encephalomyopathies; MELAS syndrome; Mitochondrial diseases; Acidosis, lactic

Introduction

Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome is a rare neurodegenerative disorder caused by the impairment of the mitochondrial respiratory chain. Approximately 80% of cases have an A to G mutation at nucleotide 3243 (A3243G) in the mitochondrial DNA molecule (*MT-TL1*), and heteroplasmy causes different mutational burdens within tis-

sues, resulting in diverse clinical phenotypes. Patients usually present with stroke-like episodes (hemianopia, cortical blindness, and dysarthria), seizures, recurrent headaches, or muscle weakness. Elevated lactate levels in blood and cerebrospinal fluid (CSF) and a change in the lactate-to-pyruvate ratio indicate respiratory chain dysfunction [1-3]. Typical magnetic resonance imaging (MRI) findings in an acute stroke-like episode of MELAS include cortical lesions. Magnetic resonance spectroscopy (MRS) is a noninvasive method that reveals metabolic information regarding brain tissue. Increased lactate levels reflect anaerobic glycolysis, and N-acetylaspartate (NAA) is a marker of neuronal integrity that represents neuronal injury associated with infarction. MRS has been used to diagnose stroke-like episodes in MELAS, with elevated lactate peaks and low NAA. Several researchers have previously reported that MRS is a useful tool for monitoring the progress of disease in patients with mitochondrial disorders [4-7]. This study aimed to evaluate the role of MRS in the initial assessment of MELAS.

Materials and Methods

The data of MELAS patients who were admitted to the pediatric neurology department at Gangnam Severance Hospital (Seoul, Korea) between January 2006 and December 2020 were reviewed. In total, 34 patients had findings compatible with the MELAS clinical diagnostic criteria, as enumerated by Bernier et al. [8]. This was a retrospective study of patients with MELAS who met the following inclusion criteria: genetically confirmed diagnosis of a pathogenic variant associated with MELAS (*MT-TL1*, m.3243A>G) and MRS performed at our center. Mutation carriers without neurological symptoms were excluded.

To identify the A3243G mutation, polymerase chain reaction amplification and restriction fragment length polymorphism analysis of total DNA extracted from leukocytes were performed. Based on this analysis, 25 patients with pathogenic mutations were included among the 34 symptomatic patients in this study. Demographic, clinical, laboratory, and MRI data were reviewed. This study was approved by the Institutional Review Board of the Gangnam Severance Hospital, Yonsei University College of Medicine(3-2017-0168). Informed consent for this retrospective study was waived by the board.

1. Mitochondrial characteristics of MELAS with A3243G

The laboratory tests consisted of blood lactate level (normal <2 mmol/L) and the lactate-to-pyruvate ratio (normal <25). Muscle biopsy samples were obtained from some patients and processed through routine morphological and histochemical staining, including periodic acid-Schiff, modified Gomori trichrome, ATPase 9.4, nicotinamide adenine dinucleotide tetrazolium reductase, and succinate dehydrogenase stains. All samples were examined for changes such as pleoconia and megaconia using electron microscopy. We investigated the initial MRS data to diagnose mitochondrial disease. MRS was performed on a GE 750 W 3.0T scanner (GE Medical Systems, Milwaukee, WI, USA) with a 16-channel head-neck combined coil including T2-weighted sequences to obtain lactate at 1.3 ppm and NAA at 2.02 ppm. The voxels were placed in the frontal and occipital gray matter and basal ganglia of the bilateral hemispheres.

The clinical severity of the patients was graded as follows: mild, self-ambulatory, with or without independence for daily activities; moderate, full-time wheelchair-bound, or partially dependent for daily activities, with the ability to engage in brief communication; and severe, bedridden, totally dependent for daily activities, or dead. All analyses were conducted using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Categorical variables were compared using the chi-square test and the Fisher exact test. Differences were considered statistically significant at a P value of <0.05.

Results

1. Demographics and mitochondrial characteristics of patients with A3243G MELAS

To evaluate the role of MRS in the initial evaluation process for the diagnosis of MELAS, this study adopted a retrospective design involving a total of 25 patients (13 boys/men and 12 girls/women) who presented with neurological symptoms and in whom a genetic analysis identified the pathogenic variant A3243G in *MT-TL1* (Table 1). The median age upon first clinical presentation was 13.3 years (range, 0.2 to 45.6), and the median time interval from the first clinical presentation to the diagnosis of MELAS was 4.8 months. The first symptoms of the disease were seizures (36%), visual impairment (28%), and motor weakness (18%). All patients exhibited neurological symptoms, followed by symptoms involving the orbital, endocrine, cardiac, auditory, psychological, myocardial, gastrointestinal, and renal systems.

The median serum lactate level was 4.3 mmol/L (range, 0.9 to 13.5), and 92% of patients had a value of 2 mmol/L or higher. An elevated lactate-to-pyruvate ratio was found in only 52% of the patients (Table 2).

2. MRI findings in patients with A3243G MELAS

MRI studies performed to assess acute neurologic episodes identified cortical lesions in 24 (96%) of the 25 patients. Seventeen patients (68%) showed old infarctions with volume loss and diffuse cerebral atrophy, and 16 patients (64%) demonstrated diffuse cere-

Table 1. Clinical characteristics of MELAS (A3243G) patients (n=25)

Characteristic	Value
Sex (male:female)	0.55
Age at first clinical presentations (yr)	14.2 ± 10.9
Age at diagnosis of MELAS (yr)	16.7 ± 10.6
Time interval from first clinical presentation to the diagnosis of MELAS (yr)	0.9 ± 1.3
Familial history of mitochondrial disease	4 (16)
Presentation symptoms at disease onset	
Seizure	9 (36)
Visual disturbance	7 (28)
Motor weakness	4 (16)
Headache	2 (8)
Loss of consciousness	2 (8)
Delayed development	1 (4)
Organ involvement	
Central nervous system	25 (100)
Endocrinologic	16 (64)
Hearing	15 (60)
Myopathy	14 (56)
Eye	13 (52)
Cardiologic	13 (52)
Psychological	11 (44)
Gastrointestinal system	9 (36)
Renal system	8 (32)

Values are presented as mean±standard deviation or number (%).
MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes.

bellar atrophy. Nine patients (36%) had lesions in the basal ganglia, and two patients (8%) had lesions in the thalamus.

3. MRS

Remarkably, the MRS results did not show statistically significant differences between patients in whom MELAS was genetically confirmed and those without genetic confirmation (Table 3). MRS alone was insufficient for identifying patients with MELAS as an initial diagnostic marker.

Increased lactate peaks were observed in 18 of 25 MRS studies (72%), and decreased NAA peaks were observed in 17 (68%) (Table 2). The acute stage was defined as clinical staging within the first 2 weeks of neurologic symptoms [9,10]. MRS studies were performed in the acute stage in 10 of the 25 patients with A3243G after neurologic episodes (seizures in three patients, visual disturbances in three patients, confusion in two patients, motor weakness in one patient, and headache in one patient). Fifteen of the 25 initial MRS studies were performed to screen patients with mitochondrial disease who did not show acute neurologic deterioration. Elevated lactate peaks were found in all 10 genetically confirmed MELAS patients in the acute stage with neurological symptoms, whereas they were found in eight of 15 patients (53%) who underwent MRS more than 2 weeks after acute episodes (P=0.011) (Fig. 1).

Table 2. Mitochondrial characteristics of MELAS (A3243G) patients (n=25)

Characteristic	Value
Serum lactate, initial (mmol/L)	4.3 (0.9–13.5)
Lactic acidosis (> 2 mmol/L)	23 (92)
Serum lactate-to-pyruvate ratio, initial	27.5 (11.3–52.8)
Increased (> 25)	13 (52)
Magnetic resonance imaging	
Infarction	24 (96)
Cortex signal abnormality	24 (96)
Diffuse cerebral atrophy	17 (68)
Cerebellar atrophy	16 (64)
Basal ganglia signal abnormality	9 (36)
Thalamus signal abnormality	2 (8)
White matter signal abnormality	19 (76)
Magnetic resonance spectroscopy	
Increased lactate peak	18 (72)
Decreased NAA	17 (68)
Muscle biopsy obtained (n = 7)	7 (28)
Light microscopic changes (+)	3 (43)
Electron microscopic changes (+)	2 (29)
Clinical severity	
Mild	15 (60)
Moderate	3 (12)
Severe	4 (16)
Death	3 (12)
Delayed development or mental retardation	17 (85)
Regression/deterioration	13 (52)
Oxygen dependency	2 (8)
Enteral tube feeding	4 (16)

Values are presented as median (interquartile range) or number (%).
MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NAA, N-acetylaspartate.

Table 3. Magnetic resonance spectroscopy findings in MELAS patients

	A3243G mutation (+)	A3243G mutation (-)	*P* value
Increased lactate peak (+)	18/25	4/9	0.138
Decreased NAA peak (+)	17/25	5/9	0.942

MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; NAA, N-acetylaspartate.

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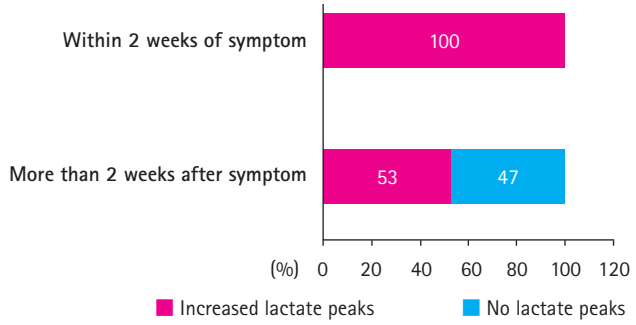

Fig. 1. Increased lactate peaks shown on magnetic resonance spectroscopy in mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS; A2343G) patients (n=25).

Discussion

In this study, we evaluated the role of MRS in the initial diagnosis of MELAS. Most initial MELAS evaluations begin with a series of biochemical studies of blood, urine, and CSF. Elevated lactate levels have long been associated with mitochondrial diseases. False elevations of plasma lactate are common because of improper specimen collection and handling. An elevated lactate-to-pyruvate ratio provides a more useful tool than serum lactate values for indicating respiratory chain dysfunction. CSF lactate levels are not influenced by the collection technique and are elevated in mitochondrial disorder patients with predominant brain manifestations.

Suomalainen et al. [11] reviewed the sensitivity (52%) and specificity (92%) of lactate measurements in patients with genetically confirmed mitochondrial disease. Blood lactate levels can be normal in MELAS patients; however, even in the presence of normal serum lactate, the CSF lactate level may be a more reliable diagnostic marker for MELAS.

Tsujikawa et al. [12] reported that the concentration of CSF lactate was closely correlated with that of brain tissue lactate obtained via MRS. Elevations of brain lactate levels are generally indicative of increased anaerobic glycolytic rates. NAA reduction reflects neuronal injury and impaired mitochondrial dysfunction after recurrent stroke-like episodes.

Previous studies have reported that brain metabolites could be evaluated using MRS to determine disease progression and perform treatment monitoring in patients with mitochondrial disorders [5,13]. Heteroplasmy results in a variety of clinical phenotypes, from asymptomatic carriers to fully symptomatic patients, as well as different mutational burdens between and within tissues. Weiduschat et al. [7] recently reported that metabolic abnormalities detected by MRS in asymptomatic A3243G carriers have potential clinical value as biomarkers of disease progression and therapeutic response. A recent study comparing the efficacy of MRS after acute neurologic symptoms in patients with acute ischemic stroke (AIS) and patients with MELAS found a lactic acid peak in only 69.2% of AIS patients, whereas a lactic acid peak was found in all patients with MELAS [14,15]. Therefore, MRS findings can reflect a lack of energy production due to impaired oxidative phosphorylation caused by nonspecific ischemia and hypoxia in the acute phase, and the relatively high sensitivity of MRS makes it a useful tool for the diagnosis of MELAS patients.

This study aimed to evaluate the clinical value of non-invasive MRS to detect brain metabolites in the initial diagnosis of genetically confirmed A3243G MELAS patients. Among 34 MELAS cases, no significant difference was found for increased lactate ($P>0.05$) or decreased NAA peaks ($P>0.05$) in MRS between the groups with and without genetic confirmation. Twenty-five of the 34 patients were diagnosed with genetically confirmed MELAS with the A3243G pathogenic mutation. Only 18 (72%) of those 25 patients had a lactate peak on initial MRS.

The detection of a lactate peak on MRS is dependent on the timing or severity of the disease. In the 25 genetically confirmed MELAS patients, the timing of the initial MRS test was divided into groups according to whether MRS was performed in the acute stage, despite the limited number of samples, and lactic acid peaks were found in all MRS data performed within 2 weeks after the first neurologic symptoms. In this study, the neurological symptoms of MELAS most commonly presented as stroke-like episodes, and elevated lactate peaks in MRS were identified in all MELAS patients in the acute stage with or without serum lactic acidosis. Elevated lactate peaks in initial MRS were not identified in patients with resolving first stroke-like symptoms, who showed symptoms at least 28 days before MRS. However, elevated lactate peaks on MRS and severe diffuse cerebral atrophy on MRI were shown in eight of 15 patients who underwent MRS more than 2 weeks after initial onset, and those patients had at least two recurrent stroke-like episodes despite the current absence of acute symptoms. Decreased NAA peaks were found in 17 of the 25 MELAS patients. The decreasing trend in NAA concentration reflects brain damage due to recurrent stroke-like attacks during prolonged periods with or without acute symptoms.

In conclusion, although initial MRS alone is not enough to definitely diagnose MELAS, it is worthwhile as a non-invasive complementary diagnostic tool along with genetic testing, as well as for monitoring the disease progress of MELAS.

Conflicts of interest

Young-Mock Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or

decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Author contribution

Conceptualization: HL and YML. Data curation: HL and JHS. Formal analysis: HL and JHN. Methodology: YML. Project administration: JHS and YML. Visualization: HL and JHN. Writing-original draft: HL. Writing-review & editing: HL and YML.

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