Original Article
Yonsei Med J 2019 Jan;60(1):98-105
https://doi.org/10.3349/ymj.2019.60.1.98

Yonsei Medical Journal
YMJ
pISSN: 0513-5796 · eISSN: 1976-2437

The Usefulness of Muscle Biopsy in Initial Diagnostic Evaluation of Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-Like Episodes

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Purpose: The disease entity mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) is characterized by an early onset of stroke-like episodes. MELAS is the most dominant subtype of mitochondrial disease. Molecular genetic testing is important in the diagnosis of MELAS. The mitochondrial DNA (mtDNA) 3243A>G mutation is found in 80% of MELAS patients. Nevertheless, molecular analysis alone may be insufficient to diagnose MELAS because of mtDNA heteroplasmy. This study aimed to evaluate whether muscle biopsy is useful in MELAS patients as an initial diagnostic evaluation method.
Materials and Methods: The medical records of patients who were diagnosed with MELAS at the Department of Pediatrics of Gangnam Severance Hospital between January 2006 and January 2017 were reviewed. The study population included 12 patients. They were divided into two subgroups according to whether the results of muscle pathology were in accordance with mitochondrial diseases. Clinical variables, diagnostic evaluations, and clinical outcomes were compared between the two groups.
Results: Of the 12 patients, seven were muscle pathology-positive for mitochondrial disease. No statistically significant difference in clinical data was observed between the groups that were muscle pathology-positive and muscle pathology-negative for mtDNA 3243A>G mutation. Additionally, the patients with weakness as the initial symptom were all muscle pathology-positive.
Conclusion: The usefulness of muscle biopsy appears to be limited to an initial confirmative diagnostic evaluation of MELAS. Muscle biopsy can provide some information in MELAS patients with weakness not confirmed by genetic testing.

Key Words: MELAS, muscle biopsy, genetic diagnosis, morphological diagnosis

INTRODUCTION

Mitochondria are organelles in cells that perform a variety of cellular metabolic functions, including the generation of most cellular energy in the form of adenosine triphosphate.[1] Mitochondrial disease is a clinically heterogeneous group of diseases, in which mitochondrial dysfunction is caused by several mutations of mitochondrial or nuclear DNA.[2]

The disease mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) is characterized by early onset of stroke-like episodes. MELAS syndrome is the most dominant subtype of mitochondrial disease.[3,4] It is one of the most frequent maternally inherited mitochondrial disorders and has been shown to be associated with an A>G transition at position 3243 of the tRNA$^{LeU(UUR)}$ gene.[5,6]

The diagnosis of MELAS is based on a combination of clinical findings, biochemical testing, muscle pathology, and molecular genetic testing. Typically, a blood sample is initially tested as part of the diagnostic evaluation in patients clinically suspected with MELAS.[7] Meanwhile, the usefulness of muscle biopsy in terms of initial diagnostic evaluation remains unknown. This study aimed to study the usefulness of muscle biopsy in MELAS patients with mitochondrial DNA (mtDNA) 3243A>G mutation as an initial diagnostic tool.

Received: May 30, 2018 **Revised:** October 21, 2018
Accepted: October 23, 2018
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•The authors have no potential conflicts of interest to disclose.

MATERIALS AND METHODS

Patients

The medical records of patients who were diagnosed with MELAS based on the diagnostic criteria of Yatsuga, et al.[2] at the Department of Pediatrics of Gangnam Severance Hospital between January 2006 and January 2017 were reviewed. Patients without mtDNA mutation were excluded from the analysis. The Institutional Review Board of Gangnam Severance Hospital in Seoul, Korea approved all procedures (3-2015-0156). Informed consent was obtained, and all methods were performed in accordance with the relevant guidelines and ethics board regulations.

Study design

The study population was divided into two subgroups according to whether the results of muscle pathology were in accordance with mitochondrial diseases or not (n=7 vs. n=5, respectively). Clinical variables, diagnostic evaluations, and clinical outcomes were compared between the two groups (Fig. 1).

Clinical characteristics, diagnostic investigations, and clinical outcomes for MELAS

Clinical data on age at onset of the first symptom, nature of the first symptom, age at diagnosis, period from the first symptoms to the last visit, and organ involvement were collected. Laboratory test results were also obtained, including serum lactic acid levels. The degree of serum lactic acidosis was defined as mild, moderate, or severe if the increase was more than the normal reference values at less than two-, three-, or more than three-fold, respectively. All patients were tested for genetic mutations involved in MELAS, including mtDNA 3243A>G mutation. Muscle biopsies from the quadriceps muscle, with which histologic, light microscopic, and electron microscopic examinations were performed. Specific findings for mitochondrial diseases under a light microscope were defined as the presence of ragged red fibers (RRF) or abnormal staining. Abnormal mitochondrial morphology was defined under the electron microscope as pleoconia and megaconia. Biochemical enzyme assay in the muscle was also performed to evaluate mitochondrial respiratory chain (MRC) enzyme activity. MRC complex defect was defined as a residual enzyme activity <10% of the reference value. Data from magnetic resonance imaging and magnetic resonance spectroscopy study were also collected.

The clinical severity of patients was defined as follows: normal, ambulatory, and independent for daily activities; mild, ambulatory, or independent for daily activities; moderate, wheelchair-bound, or partially dependent for daily activities; and severe, bedridden, totally dependent for daily activities, or expired.

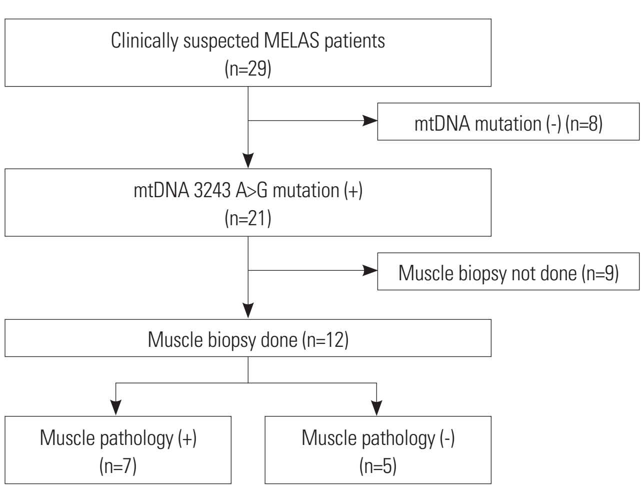

Fig. 1. Flowchart of patient inclusion. MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; mtDNA, mitochondrial DNA.

Statistical analysis

All analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used including the median and range. Differences between subgroups were evaluated using the Mann-Whitney U test (Wilcoxon rank sum test) and Fisher's exacts test. *p* values <0.05 were considered statistically significant.

RESULTS

Patient characteristics and clinical features at the last visit

A total of 21 patients was recruited in this study. Among them, 12 were male and nine were female (Table 1). The initial presenting symptoms varied, with seizures (9 of 21, 42.9%) being the most common, followed by mental change (4 patients, 19%), weakness (4 patients, 19%), visual disturbance, and ataxia. The mean age of the first symptom onset was 14.9±9.7 years. Central nervous system involvement was found in all patients, and various organs were affected as shown in Table 1. Besides central nervous system involvement, the ear was the most commonly involved organ in 16 (76.2%) patients. The majority of patients had multiple organ involvement including the gastrointestinal tract, endocrine system, eyes, and heart (71.4, 71.4, 66.7, and 57.1%, respectively).

Diagnostic evaluations

All patients were diagnosed with MELAS, which was confirmed by genetic testing, and they were all positive for mtDNA 3243A>G mutation. Increased serum lactic acid levels were observed in all patients. Magnetic resonance images of the brain revealed a variety of abnormal findings, including atrophy or abnormal signal intensities in different areas, and almost all patients (20 of 21 patients) had evidence of infarction at the time of the study. Magnetic resonance spectroscopy study data were obtained in 19 patients. The presence of lactate peak was observed in 16 patients (84.2%). Muscle biopsy was performed in 12 patients (57.1%). Table 2 shows the abnormal findings seen under light microscopy and electron microscopy, as well as MRC enzyme activity: abnormal changes specific to mitochondrial diseases under a light microscope were

Table 1. Characteristics and Clinical Features at the Last Visit

Characteristics and clinical features	Total (n=21)
Sex (male:female) (%)	12 (57.1):9 (42.9)
Age at onset of the first symptom (yr)	14.9±9.7 (0.6–37.3)
Age at onset of the first seizure (yr)	15.6±9.6 (0.6–37.3)
Time interval from first clinical presentation to the last visit (yr)	7.2±4.2 (1.4–14.4)
Initial presenting symptoms, n (%)	
Seizure	9 (42.9)
Mental change	4 (19.0)
Weakness	4 (19.0)
Visual disturbance	3 (14.3)
Ataxia	1 (4.8)
Clinical features at the last visit, n (%)	
Central nervous system	21 (100)
Respiratory system	
Frequent pneumonia	4 (19.0)
O_2 dependency	1 (4.8)
Normal	16 (76.2)
Renal system	
Abnormal kidney findings of ultrasonography	6 (28.6)
Nephrotic syndrome or proteinuria	4 (19.0)
Normal	11 (52.4)
Gastrointestinal system	
Gastroesophageal reflux disease	11 (52.3)
Enteral tube feeding	9 (42.9)
Diffuse liver disease	6 (28.6)
Gallbladder polyp or stone	4 (19.0)
Pancreatitis	2 (9.5)
Normal	6 (28.6)
Heart	
Cardiomyopathy	10 (47.6)
Wolff-Parkinson-White syndrome	6 (28.6)
Normal	9 (42.9)
Eye	
Optic atrophy	8 (38.1)
Retinopathy	4 (19.0)
Visual field defect	2 (9.5)
Ophthalmoplegia	1 (4.8)
Normal	7 (33.3)
Ear	
Hearing impairment	14 (66.7)
Hearing loss	2 (9.5)
Normal	5 (23.8)
Endocrine system	
Diabetes mellitus	13 (61.9)
Osteoporosis	4 (19.0)
Adrenal insufficiency	1 (4.8)
Normal	6 (28.6)

Table 2. Diagnostic Evaluations of MELAS (n=21)

Evaluations	n (%)
Mitochondrial DNA 3243A>G mutation (+)	21 (100)
Serum lactic acidosis	
Mildly increased (<2-fold)	5 (23.8)
Moderately increased (2–3-fold)	9 (42.9)
Severely increased (≥3-fold)	7 (33.3)
MRI obtained	21/21 (100)
Infarction	20 (95.2)
Diffuse atrophy	
Mild	8 (38.1)
Severe	10 (47.5)
Cerebellar signal abnormality	18 (85.7)
Basal ganglia signal abnormality	12 (57.1)
Thalamus signal abnormality	2 (9.5)
White matter signal abnormality	14 (66.7)
Cortex signal abnormality	20 (95.2)
MR spectroscopy obtained	19/21 (90.5)
Presence of lactate peak	16/19 (84.2)
Muscle biopsy obtained	12/21 (57.1)
Myopathic changes (+)	8/12 (66.6)
Light microscopy	
Specific findings for mitochondrial diseases	7/12 (58.3)
Nonspecific findings	1/12 (8.3)
Normal	4/12 (33.3)
Electron microscopy	
Pleoconia	4/12 (33.3)
Megaconia	4/12 (33.3)
Normal	8/12 (66.7)
MRC complex enzyme assay obtained	10 (47.6)
MRC complex I defect	8/10 (80.0)
MRC complex IV defect	1/10 (10.0)
MRC complex I and IV defect	1/10 (10.0)

MELAS, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes; MRC, mitochondrial respiratory chain.

Table 3. Clinical Outcomes at Last Visit

Clinical outcomes	Total (n=21)
Number of stroke-like episodes	3.5±2.9 (0–10)
Delayed development or mental retardation, n (%)	16 (76.2)
Clinical severity at last outpatient clinic, n (%)	
Normal (ambulatory and independent for daily activities)	6 (28.6)
Mild (ambulatory or independent for daily activities)	3 (14.3)
Moderate (WC bound and/or partially dependent for daily activities)	4 (19.0)
Severe (bedridden, totally dependent for daily activities or expired)	8 (38.1)

WC, wheelchair.

https://doi.org/10.3349/ymj.2019.60.1.98

seen in seven of the 12 patients (58.3%); electron microscopic changes with pleoconia or megaconia were noted in four of the 12 patients (33.3%). Biochemical enzyme assay in the muscle tissue revealed deficiency of MRC complex I in nine of 10 patients (90%) (Table 2).

Table 4. Comparison of General Characteristics and Clinical Features between Subgroups

Characteristics and clinical features	Muscle pathology (+) (n=7)	Muscle pathology (-) (n=5)	*p* value
Sex, n (%)			
Male	4 (57.1)	3 (60.0)	0.689
Female	3 (42.9)	2 (40.0)	
Age at onset of the first symptom (months)	94 (17–448)	134 (67–426)	0.639
Age at onset of the first seizure (months)	132 (55–448)	134 (67–426)	1.000
Time interval from the first clinical presentation to the last visit (months)	109 (23–137)	114 (25–136)	0.755
Initial presenting symptoms, n (%)			
Seizure	3 (42.9)	2 (40.0)	0.689
Mental change	0 (0)	2 (40.0)	0.152
Weakness	4 (57.1)	0 (0)	0.071
Ataxia	0 (0)	1 (20.0)	0.417
Clinical features at the last visit, n (%)			
Central nervous system	7 (100)	5 (100)	-
Respiratory system			
Frequent pneumonia	2 (28.6)	1 (20.0)	0.636
Oxygen dependency	1 (14.3)	0 (0)	0.583
Normal	4 (57.1)	4 (80.0)	-
Renal system			
Abnormal kidney findings of ultrasonography	4 (57.1)	1 (20.0)	0.247
Nephrotic syndrome or proteinuria	2 (28.6)	1 (20.0)	0.636
Normal	1 (14.3)	3 (60.0)	-
Gastrointestinal system			
Gastroesophageal reflux disease	5 (71.4)	3 (60.0)	0.576
Enteral tube feeding	4 (57.1)	3 (60.0)	0.689
Diffuse liver disease	3 (42.9)	2 (40.0)	0.689
Gallbladder polyp or stone	1 (14.3)	2 (40.0)	0.364
Pancreatitis	1 (14.3)	0 (0)	0.583
Normal	2 (28.6)	0 (0)	-
Heart			
Cardiomyopathy	4 (57.1)	3 (60.0)	0.576
Wolff-Parkinson-White syndrome	2 (28.6)	2 (40.0)	0.636
Normal	3 (42.9)	1 (20.0)	-
Eye			
Optic atrophy	2 (28.6)	2 (40.0)	0.576
Retinopathy	3 (42.9)	0 (0)	0.159
Visual field defect	0 (0)	1 (20.0)	0.417
Ophthalmoplegia	1 (14.3)	0 (0)	0.583
Normal	1 (14.3)	2 (40.0)	-
Ear			
Hearing impairment	6 (85.7)	4 (80.0)	0.682
Hearing loss	1 (14.3)	0 (0)	0.583
Normal	0 (0)	1 (20.0)	-
Endocrine system			
Diabetes mellitus	3 (42.9)	4 (80.0)	0.247
Osteoporosis	2 (28.6)	1 (20.0)	0.636
Adrenal insufficiency	0 (0)	1 (20.0)	0.417
Normal	3 (42.9)	0 (0)	-

Clinical outcomes

The mean time interval from the first clinical presentation to the last visit at our institute was 7.2±4.2 years, and the number of stroke like episodes averaged during the study duration was 3.5±2.9 (range 0 to 10). Of the 21 patients, 16 patients (76.2%) exhibited delayed development or mental retardation, and 12 patients (57.1%) had moderate to severe impairment in daily living functions. Our data showed that clinical severities were quite variable (Table 3).

Analysis of general characteristics, clinical features, diagnostic evaluations, and clinical outcomes by subgroup

Patients with weakness as the initial symptom demonstrated

Table 5. Comparison of Diagnostic Evaluations between Subgroups

Evaluations	Muscle pathology (+) (n=7)	Muscle pathology (-) (n=5)	*p* value
Mitochondrial DNA 3243A>G mutation (+), n (%)	7 (100)	5 (100)	-
Serum lactic acidosis, n (%)			
Mildly increased (<2-fold)	1 (14.3)	1 (20.0)	0.682
Moderately increased (2–3-fold)	1 (14.3)	3 (60.0)	0.152
Severely increased (≥3-fold)	5 (71.4)	1 (20.0)	0.121
MRI obtained, n (%)			
Infarction	6 (85.7)	4 (80.0)	0.583
Diffuse atrophy			
Mild	1 (14.3)	1 (20.0)	0.682
Severe	5 (71.4)	4 (80.0)	0.636
Cerebellar signal abnormality	6 (85.7)	4 (80.0)	0.682
Basal ganglia signal abnormality	4 (57.1)	3 (60.0)	0.689
Thalamus signal abnormality	1 (14.3)	1 (20.0)	0.682
White matter signal abnormality	5 (71.4)	3 (60.0)	0.576
Cortex signal abnormality	6 (85.7)	5 (100)	0.583
MR spectroscopy obtained, n (%)	7 (100)	4 (80.0)	
Presence of lactate peak	7/7 (100)	4/4 (100)	0.417
Muscle biopsy obtained, n (%)	7 (100)	5 (100)	-
Myopathic changes (+), n (%)	7 (100)	1 (20.0)	-
Light microscopy			
Specific findings for mitochondrial diseases	7 (100)	0 (0)	<0.001
Nonspecific findings	0 (0)	1 (20.0)	0.417
Normal	0 (0)	4 (80.0)	-
Electron microscopy			
Pleoconia	4 (57.1)	0 (0)	0.071
Megaconia	4 (57.1)	0 (0)	0.071
Normal	3 (42.9)	5 (100)	-
MRC complex enzyme assay, n (%)	6 (85.7)	4 (80.0)	
MRC complex I defect	6/6 (100)	2/4 (50)	0.152
MRC complex IV defect	0 (0)	1/4 (25.0)	0.417
MRC complex I and IV defect	0 (0)	1/4 (25.0)	0.417

MRC, mitochondrial respiratory chain.

Table 6. Comparison of Clinical Outcomes between Subgroups

Clinical outcomes at last visit	Muscle pathology (+) (n=7)	Muscle pathology (-) (n=5)	*p* value
Number of stroke-like episodes	4.0±3.3 (1–10)	4.0±2.9 (2–9)	0.876
Delayed development or mental retardation, n (%)	7 (100)	4 (80.0)	0.417
Clinical severity at last outpatient clinic, n (%)			
Normal (ambulatory and independent for daily activities)	1 (14.3)	1 (20.0)	0.682
Mild (ambulatory or independent for daily activities)	0 (0)	1 (20.0)	0.417
Moderate (WC bound or partially dependent for daily activities)	2 (28.6)	0 (0)	0.318
Severe (bedridden, totally dependent for daily activities or expired)	4 (57.1)	3 (60.0)	0.689

WC, wheelchair.

https://doi.org/10.3349/ymj.2019.60.1.98

positive muscle pathology, although the difference was statistically insignificant (p=0.071). No significant trends were observed concerning general characteristics or clinical features according to muscle pathology, nor were there any significant differences in the severity of serum lactic acidosis or neuroimaging studies and organ involvement (Tables 4 and 5). Dividing the patients into subgroups according to muscle pathology, we found that differences in light microscopic changes were statistically significant, while electron microscopic changes were similar (p<0.001, p=0.071, respectively). No meaningful differences were found in terms of the parameters of the number, development, and clinical severity of stroke-like episodes (Table 6).

DISCUSSION

Most of the current diagnostic criteria on mitochondrial disease were developed prior to the recent expansion of molecular genetic knowledge.[8,9] These criteria are based on combinations of clinical, laboratory, pathologic, biochemical, and genetic findings.[10] Given the complexity of mitochondrial diseases, their clinical manifestations are extremely heterogeneous, and the various combinations of organ involvement have led to defining mitochondrial syndromes according to the presentations thereof: some are well known for their acronyms, such as MELAS and myoclonic epilepsy with RRF.[1] However, with outstanding accuracy, molecular diagnosis is gaining popularity, and with further development in diagnostic technology, muscle biopsy has generally come to be considered less useful in MELAS patients confirmed by genetic testing. Our study is unique and meaningful because few studies have sought to assess the usefulness of muscle biopsy in mitochondrial diseases, such as MELAS.

MELAS syndrome was first described in 1984 by Pavlakis, et al.[4] Later, Hirano, et al.[11] defined the three almost invariant criteria of MELAS: 1) stroke-like episode before the age of 40 years; 2) encephalopathy characterized by seizures, dementia, or both; and 3) lactic acidosis, presence of RRFs, or both. The diagnosis is confirmed if at least two of the following criteria were also present: 1) normal early development, 2) recurrent headaches, and 3) recurrent vomiting episodes. Recently, the MELAS study committee in Japan published other diagnostic criteria by which the diagnosis is considered definitive with at least two category A criteria (headaches with vomiting, seizures, hemiplegia, cortical blindness, and acute focal lesions in neuroimaging) and two category B criteria (high plasma or cerebrospinal fluid lactate, mitochondrial abnormalities in muscle biopsy, and a MELAS-related gene mutation).[6] Molecular genetic testing is also important in the diagnosis of MELAS. The mtDNA 3243A>G mutation in the *MT-TL1* gene encoding $tRNA^{Leu(UUR)}$ is found in 80% of MELAS patients.[5,12] Additional mtDNA mutations have been shown to be associated with MELAS, such as the *MT-TL1* pathogenic variants (mitochondrial DNA 3271 T>C and mitochondrial DNA 3252 A>G mutations).[13] Currently, over 30 mtDNA gene mutations have been found to be associated with this syndrome (http://www.ncbi.nlm.nih.gov/omim/540000). Our data were consistent with previous studies in terms of prevalence of the mtDNA 3243A>G mutation.[2] Molecular genetic testing was performed in all patients, and they were all (100%) positive for mtDNA 3243A>G mutation. No other mutations were noted. The results of the present study correspond with an earlier study that reported that the primary considerable and common gene is mtDNA 3243A>G mutation. The mtDNA 3243A>G mutation in the *MT-TL1* gene has a wide phenotypic spectrum, including MELAS, chronic progressive external ophthalmoplegia, maternally inherited deafness and diabetes, and Leigh syndrome. Other reported features include isolated myopathy, cardiomyopathy, seizures, migraine, ataxia, cognitive impairment, bowel dysmotility, and short stature.[14,15] The extreme variability in the clinical phenotype is due to the proportion of mutated mtDNA or the number of wild-type mtDNA within tissues.[16,17] Detection of mtDNA point mutation without quantification alone has been generally accepted to be insufficient in the diagnosis of MELAS because of mtDNA heteroplasmy. The heteroplasmy of mtDNA makes the detection of mtDNA point mutation alone and without quantification insufficient to diagnose MELAS.

Before the development of newer molecular genetic testing, muscle biopsy was thought to be the best method to obtain accurate diagnosis of mitochondrial diseases, MELAS in particular. The diagnosis of MELAS is often confirmed by the presence of RRF on succinate dehydrogenase histochemical stain, as a result of diseased mitochondrial aggregates in the subsarcolemmal areas of muscle fibers.[10,18,19] Muscle biopsy also provides muscle tissue samples for morphological, biochemical, and molecular studies. In the current study, no statistically significant difference was observed between MELAS patient harboring 3243A>G point mutation who were positive for muscle pathology and those who were negative for muscle pathology. On the basis of this finding, we deemed the usefulness of muscle biopsy to be limited at initial confirmative diagnosis of MELAS. Additionally, the patients with weakness as the initial symptom of MELAS were all positive for muscle pathology. While it is assumed that muscle pathology can provide some information to MELAS patients with weakness, no statistically significant relationship was found between the groups that were positive for muscle pathology and negative for muscle pathology (p=0.071). Nevertheless, these findings may reflect the difficulty of obtaining a statistical correlation because of the limited number of patients in each subgroup. Moreover, myopathy has long been recognized and well described in MELAS and several other mitochondrial diseases.[18] In a prospective cohort of MELAS patients in Japan, of 96 patients, 36 (37.5%) patients had muscle weakness at onset, and 40 (41.7%)

experienced muscle weakness over the entire course.[2] The importance of muscle pathology in a patient suspected with MELAS syndrome should not be overlooked, particularly in a patient whose predominant clinical symptoms include muscle weakness.[3]

MELAS patients harboring point mutation involve a high frequency of RRF.[20] However, no studies investigating the usefulness of muscle biopsy in MELAS patients diagnosed by molecular analysis have been conducted. Previous studies have reported that the accumulation of mitochondria in muscle fibers, which were composed of typical RRF, is found in up to 97% of MELAS patients.[20,21] Nevertheless, several points should be considered in regards to the limitations of muscle biopsy. The procedure is invasive and requires sedation when dealing with children. On occasion, the pathologist is only able to conclude that the specimen provides findings consistent with an unspecified myopathy. This result may occur for a variety of reasons, including cases where the obtained tissue may not have been taken from a disease-fulminant region or the volume of muscle tissue is not enough.[22] Moreover, a normal reference value of muscle tissue based on age still remains unknown.[10,19] Notwithstanding, muscle biopsy has been performed in the routine analysis for mitochondrial disease when the diagnosis cannot be confirmed with genetic testing.[10]

This study was retrospective in nature, and it has some limitations. Because of disease rarity, the size of the study population was small, which may imply that the results are not sufficient for making generalized interpretations. From the results, the usefulness of muscle biopsy was shown to be limited in making the initial confirmative diagnosis of MELAS. Muscle biopsy was not able to provide additional information on MELAS patients when already diagnosed through genetic testing. However, muscle biopsy could provide some information if genetic testing cannot confirm a diagnosis in clinically suspected MELAS patients with weakness, which may be a symptom of myopathy. In short, though a genetic test should be the first choice to diagnose MELAS, muscle biopsy should be considered as an additional choice in clinically suspected MELAS patient with weakness not confirmed by genetic testing. In order to provide a more generalized guideline, studies on larger numbers of patients are needed to better delineate the usefulness of muscle biopsy in MELAS. Mitochondrial disease is heterogeneous in general, and each of the mitochondrial syndromes tend to display their own specific clinical manifestations. These characteristics warrant continuous study, and whether previous diagnostic tools are in fact the best methods for each specific type of syndrome needs to be verified.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI16C0673).

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