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원 저

Leigh 증후군 환자의 임상적 생화학적 진단

연세대학교 의과대학 소아청소년과[1], 고잔 연세 소아청소년과[2], 연세대학교 의과대학 병리과[3]

이선호[1] · 전미나[1] · 이현주[1] · 박대영[2] · 김세훈[3] · 이영목[1]

Clinical and Biochemical Diagnosis in Children with Leigh Syndrome

Sun Ho Lee, M.D.[1], Mina Jeon, M.D.[1], Hyun Joo Lee, M.D.[1], Dae Young Park, M.D.[2]
Se Hoon Kim, M.D., Ph.D.[3], Young-Mock Lee, M.D., Ph.D.[1]

Departments of Pediatrics[1], Yonsei University College of Medicine, Seoul, Korea
Kojan Yonsei Pediatric Clinic[2], Ansan, Korea
Departments of Pathology[3], Yonsei University College of Medicine, Seoul, Korea

Purpose: Deficits of the respiratory chain are reported to be the major cause of Leigh syndrome is said to be the underlying causes. The need for biochemical diagnosis to draw more accurate diagnosis or prognosis to support treatments is rapidly increasing. This study tried to analyze the aspects of clinical characteristics and biochemical diagnosis of mitochondrial respiratory chain complex (MRC) defect in Leigh syndrome, using methods of biochemical enzyme assay.

Methods: We included total number of 47 patients who satisfied the clinical criteria of Leigh syndrome and confirmed by biochemical diagnosis. All those patients went through muscle biopsy to perform biochemical enzyme assay to analyze MRC enzyme in order to find the underlying cause of Leigh syndrome.

Results: MRC I defect was seen in 23 (48.9%) cases taking the first place and MRC IV defect in 15 (31.9%) following it. There were 9 (19.2%) cases of combined MRC defect. Combined cases of type I and IV were detected in 7 (14.9%) patients while type I and V in 2 (4.3%). The onset age of symptom was less than 1 year old in 28 (59.6%). The most common early symptom, observed in 23 (48.9%), was delayed development, but there were other various neurological symptoms observed as well. In regard with the disease progression, 35 (74.5%) patients showed slowly progressive course, the one that progressed continuously but slowly over 2 years of period. As for Maximum motor development, 22 (46.8%) were bed-ridden state, most of them suffering serious delayed development. Patients showed various symptoms with different organs involved, though neuromuscular involvement was most prominent. Delayed development was seen in all cases. Multifocal lesion in brain MRI study was seen in 36 (76.6 %) cases, taking a greater percentage than 11 (23.4%) cases with single lesion. In MR spectroscopy study, the characteristic lactate peak of mitochondrial disease was identified in 20 (42.6%) patients.

Conclusions: Further analysis of clinical and biochemical diagnosis on more extended group of patients with Leigh syndrome will enable us to improve diagnostic precision and to understand the natural course of mitochondrial disease.

Key words: Mitochondria, Leigh syndrome, Respiratory chain

Introduction

Leigh syndrome is known as the most representative mitochondrial disease involving abnormal

책임저자: 이영목, 서울 강남구 언주로 211
연세대학교 의과대학 강남세브란스병원 소아청소년과
Tel: 02)2019-3350, Fax: 02)3461-9473
E-mail: ymleemd@yuhs.ac

energy production. It shows characteristic lesion in the brain, especially in areas such as the basal ganglia, diencephalon, cerebellum, or brainstem and clinically takes a progressive natural course [1, 2]. Leigh syndrome generally starts during infancy or early childhood. Most are fatal cases and patients usually expire before the age of 5 years old. Clinically, progressive decline of central nervous system function is the most crucial symptom involved[3, 4]. But other various symptoms like psychomotor delay, weakness, hypotonia, and ataxia et al. are not difficult to observe, either. Organ involvement other than neuromuscular system occurs as well[5, 6].

Deficits of the respiratory chain, coenzyme Q, or the pyruvate dehydrogenase complex are reported to be the cause of Leigh syndrome, and mutations of the genes encoding subunits of the respiratory chain or assembly factors of respiratory chain complexes are said to be the underlying cause[7]. Thus, biochemical enzyme assay enables one to confirm the cause in Leigh syndrome.

Nonetheless, it is practically very difficult to find thoroughly all those kinds of clinical characteristics and biochemical abnormalities in every patient suspected of having Leigh syndrome. And even if one did, the results can sometimes be discordant[8]. Till today, diagnosing Leigh syndrome is done mainly based on its characteristic clinical symptoms and neuroimaging studies. The need for biochemical diagnosis to draw more accurate diagnosis or prognosis to support treatments is rapidly increasing. This study tried to analyze the aspects of clinical characteristics and biochemical diagnosis of mitochondrial respiratory chain complex (MRC) defect, the most widely known cause of Leigh syndrome, using methods of biochemical enzyme assay.

Materials and Methods

We included total number of 47 patients who had visited the pediatric neurology department in Gangnam Severance Hospital and Severance Children's Hospital between March 2010 and December 2014 and who satisfied the clinical criteria of Leigh syndrome retrospectively. Clinical criteria of Leigh syndrome are as following: (1) progressive neurological disease with motor and intellectual developmental delay; (2) signs and symptoms of brainstem and/or basal ganglia disease; (3) raised lactate levels in blood and/or cerebrospinal fluid (CSF); and (4) one or more of the following: (a) characteristic features of Leigh syndrome on neuroimaging (symmetrical hypodensities in the basal ganglia on computed tomography or hyperintense lesions on T'2–weighted magnetic resonance imaging (MRI), (b) typical neuropathological changes at postmortem, or (c) typical neuropathology in a similarly affected sibling[9]. All those 47 patients went through muscle biopsy to perform biochemical enzyme assay to analyze MRC enzyme in order to find the underlying cause of Leigh syndrome.

Skeletal muscle was assayed by modifications of published methods and sample preparation was done at 0 to 4℃[10–12]. Respiratory chain enzymes were assayed at 30℃ using a spectrophotometer on the same day that the tissue was homogenized. Evaluation of MRC dysfunction was performed by analyzing the activities of reduced nicotinamide adenine dinucleotide–coenzyme Q (CoQ) reductase (complex I), succinate–CoQ reductase (complex II), succinatecytochrome c reductase (complexes II–III), cytochrome c reductase (complex III), cytochrome c oxidase (complex IV), oligomycin–

sensitive ATPase (complex V) and citrate synthase assessed from isolated mitochondria in muscle tissue through use of standard spectrophotometric assays as described by Rustin et al[12]. We defined an MRC defect as reduction of residual enzyme activity below 20% of controls.

Results

1. Biochemical enzyme assay of MRC (Table 1)

Most cases, 38 (80.9%) to be exact, had single MRC defect. MRC I defect was seen in 23 (48.9 %) cases taking the first place and MRC IV defect in 15 (31.9%) following it. There were 9 (19.1%) cases of combined MRC defect. Combined cases of type I and IV were detected in 7 (14.9 %) patients while type I and V in 2 (4.3%).

2. Cilinical characteristics of Leigh syndrome patients with MRC defect (Table 2)

Sex ratio of 47 patients with MRC defect in biochemical enzyme assay was 1.14:1. The onset age of symptom was less than 1 year old in 28 (59.6%), neonatal period in 3 (6.4%), and after 5 years of age in 2 (4.2%) cases. The most common early symptom, observed in 23 (48.9%), was delayed development, but there were other various neurological symptoms observed as well. In regard with the disease progression, 2 (4.2%) patients followed fulminant course which lead to expire within 6 months of initial presentation. Three (6.4%) followed rapidly progressive course which showed rapid deterioration that brought expire within 2 years, and 35 (74.5%) showed slowly progressive course, the one that progressed continuously but slowly over 2 years of period. There were 7 (14.9%) patients who followed chronic stable course besides them[13]. There were 7 (14.9%) expire cases and 15 (31.9%) which needed continuous ventilator care. As for Maximum motor development, 22 (46.8%) were bed-ridden state, most of them suffering serious de-

Table 1. Results of Biochemical Enzyme Assay for MRC (N=47)

MRC function		No. of patients (%)
Abnormal	I defect	23 (48.9)
	IV defect	15 (31.9)
	I+IV defect	7 (14.9)
	I+V defect	2 (4.3)
Total		47 (100.0)

Table 2. Clinical Characteristics of Leigh Syndrome Patients with MRC Defect (N=47)

Characteristics		No. of patients (%)
Symptom onset age (years)	Neonate	3 (6.4)
	>1 month, =<1 year	25 (53.2)
	>1 year, =<5 years	17 (36.2)
	>5 years	2 (4.2)
Initial symptom	Delayed development	23 (48.9)
	Seizure	10 (21.3)
	Hypotonia	5 (10.6)
	Ataxia	4 (8.5)
	Ptosis	2 (4.2)
	Lethargy	2 (4.2)
	Spasticity	1 (2.1)
Disease course	Fulminant	2 (4.2)
	Rapidly progressive	3 (6.4)
	Slowly progressive	35 (74.5)
	Chronic stable	7 (14.9)
Maximum motor development	Bed-ridden	22 (46.8)
	Sitting	3 (6.4)
	Standing	2 (4.2)
	Walking by support	7 (14.9)
	Incomplete gait	13 (27.8)
Continuous ventilator care		15 (31.9)
Expire cases		7 (14.9)
Abnormal family history		9 (19.1)
Birth history	Prematurity	6 (12.8)
	Low birth weight	9 (19.1)
	Severe asphyxia	3 (6.4)
	Mild asphyxia	9 (19.1)

layed development. There were 12 (25.5) with the birth history of asphyxia.

3. The clinical features of organ involvement in Leigh syndrome patients with MRC defect (Table 3)

Patients showed various symptoms with different organs involved, though neuromuscular involvement was most prominent. Delayed development was seen in all cases. Hypotonia was seen in 36 (76.6%) patients and spasticity in 2 6 (55.3%). Twenty nine (61.7%) had seizure episodes while involuntary movement (46.8%), ataxia (31.9%), and dystonia (19.1%) were reported as well. Nystagmus was the most common ophthalmologic finding, noted in 14 (29.8%) patients. Ophthalmoplegia (14.9%), retinopathy (14.9%), ptosis (10.6 %), and strabismus (10.6%) were seen in others. Problems related with oral intake and failure to thrive was also observed. Poor feeding was seen in 36 (76.6%) and unexpected vomiting in 38 (80.9%) patients. Among them, 18 (38.3%) required continuous Levin tube feeding and 6 (12.8 %) even required gastrostomy feeding. Respiratory problem appeared in 17 (36.1%) cases. Cardiac involvements were seen in 11 (23.4%) cases. Hepatic, renal, and auditory system involvements were also observed.

Table 3. Clinical Features of Organ Involvement in Leigh Syndrome Patients with MRC Defect (N=47)

Clinical features		No. of patients (%)
Developmental delay		47 (100.0)
Hypotonia	Severe	19 (40.4)
	Moderate	6 (12.8)
	Mild	11 (23.4)
Spasticity	Severe	8 (17.0)
	Moderate	5 (10.6)
	Mild	13 (27.7)
Dystonia		9 (19.1)
Involuntary movement		22 (46.8)
Ataxia		15 (31.9)
Seizure		29 (61.7)
Ocular symptom	Optic atrophy	2 (4.2)
	Retinopathy	7 (14.9)
	Nystagmus	14 (29.8)
	Ophthalmoplegia	7 (14.9)
	Ptosis	5 (10.6)
	Strabismus	5 (10.6)
Poor feeding	Severe	17 (36.1)
	Moderate	10 (21.3)
	Mild	9 (19.1)
Unexpected vomiting		38 (80.9)
Levin tube feeding		18 (38.3)
Gastrostomy feeding		6 (12.8)
Failure to thrive	Severe	18 (38.3)
	Moderate	8 (17.0)
	Mild	11 (23.4)
Hepatic involvement		7 (14.9)
Cardiac involvement		11 (23.4)
Renal involvement		3 (6.4)
Respiratory problem		17 (36.1)
Auditory involvement		5 (10.6)

4. Brain MRI and MR spectroscopy findings in Leigh syndrome patients with MRC defect (Table 4)

Multifocal lesion in brain MRI study was seen in 36 (76.6%) cases, taking a greater percentage than 11 (23.4%) cases with single lesion. Basal

Table 4. Brain MRI and MR spectroscopy findings in Leigh syndrome patients with MRC defect (N=47)

Location of abnormalities	No. of patients (%)
Involvement pattern	
Multiple lesions	36 (76.6)
Single lesion	11 (23.4)
MRI	
Basal ganglia	47 (100.0)
Diffuse cerebral atrophy	25 (53.2)
Brain stem	19 (40.4)
Cerebellum	11 (23.4)
Thalamus	19 (40.4)
MR spectroscopy	
Lactate peak	20 (42.6)

ganglia lesion was observed in all patients. Diffuse atrophy was seen in 25 (53.2%) cases. Brain stem and thalamus lesion was seen in 19 (40.4%) patients respectively. In MR spectroscopy study, the characteristic lactate peak of mitochondrial disease was identified in 20 (42.6%) patients.

Discussion

Leigh syndrome is associated with heterogeneous, biochemical abnormalities in the mitochondria of the skeletal muscle, detectable in about 50% of affected patients[6, 14, 15]. This certainly implies the difficulty of diagnosing mitochondrial diseases. However, like other clinical syndromes that later get classified as specific diseases after their exact pathogenesis are understood and more definite diagnostic criteria are introduced, mitochondrial disorder is taking the similar path from being diagnosed clinical criteria by biochemical methods. The study results that we obtained show quite similar rates of diagnosis with those of previously reported biochemical diagnosis[16, 17]. These sophisticated types of diagnostic tools could support in predicting disease progression and prognosis, thus enable us to make a better judgment on treatments provided. No need to say that their assistance will be more needed in the future.

The natural course of Leigh syndrome has usually been characterized as sudden progressive neurological symptoms leading to early expire in many cases[5, 6]. Interestingly, during our study many patients rather seem to take a slow progressive course of a degenerative disease. It seems that the recent increased attentions on mitochondrial disorder have made improvements in the diagnostic skills and facilitated to make earlier diagnosis. Although no causal treatment is available for Leigh syndrome generally, the introductions of various possible drugs and improved general treatment should also be considered as the reason for the difference in the disease course. The patients we included in the study were continuously treated with drugs such coenzyme-Q, L-carnitine, and vitamins and checked for other organ involvement after being diagnosed[5, 18, 19]. Close monitoring of patients with respiratory disturbances, and the application of tests to assess brainstem function may prevent sudden death in early-onset Leigh syndrome[20].

There have been studies analyzing clinical symptoms based on biochemical diagnosis, but most were done with limited number of cases. Few were related with specific mitochondrial disease group such as Leigh syndrome. Previous reports had stated that no certain objective correlation between clinical symptoms and abnormalities in biochemical assay was observed[21]. The weakness of this study is that we did not study all the genes known to cause Leigh syndrome. Further gene analysis on more extended group of patients with Leigh syndrome will enable us not only to improve diagnostic precision but to understand mitochondrial disease one step further as well by revealing the correlation between its phenotypes and genotypes.

It is still rather a challenging work to diagnose and evaluate mitochondrial diseases precisely. However, sophisticated types of diagnostic tools such as biochemical enzyme assay and molecular analysis could give us a hand in predicting disease progression and prognosis, and enable us to make better choices when treating a patient.

References

1) Leigh D. Subacute necrotizing encephaloinyelopathy in an infant. J Neurol Neurosurg Psychiatry 1951; 14:216-21.
2) Pincus JH. Subacute necrotizing encephalomyelopathy (Leigh's disease): a consideration of clinical features and etiology. Dev Med Child Neurol 1972; 14:87-101.
3) van Erven PM, Cillessen JP, Eekhoff EM, Gabreëls FJ, Doesburg WH, Lemmens WA, et al. Leigh syndrome, a niitochondrial encephalo(myo)pathy. A review of the literature. Clin Neurol Neurosurg 1987;89:217-30.
4) van Erven PM, Gabreëls FJ, Ruitenbeek W, Renier WO, Fischer JC. Mitochondrial encephalomyopathy. Association with an NADH dehydrogenase deficiency. Arch Neurol 1987;44:775-8.
5) Piao YS, Tang GC, Yang H, Lu DH. Clinico-neuropathological study of a Chinese case of familial adult Leigh syndrome. Neuropathology 2006;26: 218-21.
6) Bénit P, Slama A, Cartault F, Giurgea I, Chretien D, Lebon S et al. Mutant NDUFS3 subunit of mitochondrial complex I causes Leigh syndrome. J Med Genet 2004;41:14-7.
7) Finsterer J. Leigh and Leigh-like syndrome in children and adults. Pediatr Neurol 2008;39:223-35.
8) Kirby DM, Boneh A, Chow CW, Ohtake A, Ryan MT, Thyagarajan D et al. Low mutant load of mitochondrial DNA G13513A mutation can cause Leigh's disease. Ann Neurol 2003;54:473-8.
9) Rahman S, Blok RB, Dahl HH, Danks DM, Kirby DM, Chow CW, et al. Leigh syndrome: Clinical features and biochemical and DNA abnormalities. Ann Neurol 1996;39:343-51.
10) Zheng X, Shoffner JM, Voljavec AS, Wallace DC. Evaluation of procedures for assaying oxidative phosphorylation enzyme activities in mitochondrial myopathy muscle biopsies. Biochim Biophys Acta 1990;1019:1-10.
11) Bindoff LA, Desnuelle C, Birch-Machin MA, Pellissier JF, Serratrice G, Dravet C, et al. Multiple defects of the mitochondrial respiratory chain in a mitochondrial encephalopathy (MERRF)-a clinical, biochemical and molecular study. J Neurol Sci 1991; 102:17-24.
12) Rustin P, Chretien D, Bourgeron T, Gérard B, Rötig A, Saudubray JM, et al. Biochemical and molecular investigations in respiratory chain deficiencies. Clin Chim Acta 1994;228:35-51.
13) Skladal D, Sudmeier C, Konstantopoulou V, Stöckler-Ipsiroglu S, Plecko-Startinig B, Bernert G, et al. The Clinical Spectrum of Mitochondrial Disease in 75 Pediatric Patients. Clin Pediatr 2003;42:703-10.
14) Jareño NM, Fernández-Mayoralas DM, Silvestre CP, Cortés BM, Pérez MU, Campos-Castelló J. 3-Methylglutaconic aciduria type 4 manifesting as Leigh syndrome in 2 siblings. J Child Neurol 2007; 22:218-21.
15) Makino M, Horai S, Goto Y, Nonaka I. Confirmation that a T-to-C mutation at 9176 in mitochondrial DNA is an additional candidate mutation for Leigh's syndrome. Neuromuscul Disord 1998;8: 149-51.
16) Martín MA, Blázquez A, Gutierrez-Solana LG, Fernández-Moreira D, Briones P, Andreu AL, et al. Leigh syndrome associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFS1 gene. Arch Neurol 2005;62:659-61.
17) Bénit P, Chretien D, Kadhom N, de Lonlay-Debeney P, Cormier-Daire V, Cabral A, et al. Large-scale deletion and point mutations of the nuclear NDUFV1 and NDUFS1 genes in mitochondrial complex I deficiency. Am J Hum Genet 2001; 68:1344-52.
18) Van Maldergem L, Trijbels F, DiMauro S, Sindelar PJ, Musumeci O, Janssen A, et al. Coenzyme Q-responsive Leigh's encephalopathy in two sisters. Ann Neurol 2002;52:750-4.
19) López LC, Schuelke M, Quinzii CM, Kanki T, Rodenburg RJ, Naini A, et al. Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. Am J Hum Genet 2006;79:1125-9.
20) Ostergaard E, Hansen FJ, Sorensen N, Duno M, Vissing J, Larsen PL, et al. Mitochondrial encephalomyopathy with elevated methylmalonic acid is caused by SUCLA2 mutations. Brain 2007;130: 853-61.
21) Martin E, Burger R, Wiestler OD, Caduff R, Boltshauser E, Boesch C. Brainstem lesion revealed by MRI in a case of Leigh's disease with respiratory failure. Pediatr Radiol 1990;20:349-50.