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Clinical and Laboratorial Characteristics of Korean Children with Mitochondrial Respiratory Chain Defect

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= 국문요약 =

신경학적 증상을 동반한 사립체 호흡 연쇄 복합체 결함 환자에 대한 임상적 분석

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목 적 : 사립체 호흡 연쇄 효소 복합체 결함 환자에서 신속하고 정확한 진단에 도움을 주고 효과적인 치료가 가능하도록 하기 위해 임상 양상과 검사 소견을 조사하였다.

방 법 : 근육조직을 이용하여 시행한 사립체 호흡 연쇄 효소 복합체에 대한 분광광도분석 결과 결함이 확인된 28명의 환자를 대상으로 후향적으로 여러 가지 임상 자료와 검사 결과를 분석하였다.

결 과 : 환자의 평균 연령은 6.67±4.44세이었고, 남녀비는 1.15:1 이었다. 18명(64.3%)이 사립체 호흡 연쇄 효소 복합체 I 결함이었고, 8명(28.6%)이 VI 결함이었으며, II 결함이 1명 있었고, I 과 IV 결함이 같이 있는 경우가 1명 있었다. 8명(28.6%)이 임상적으로 Leigh 증후군이었고, MELAS, Kearns-Sayre 증후군, Alpers 증후군이 각각 1명씩 있었으나, 대부분은 특정 질환의 기준에 부합되지 않았다. 간질은 동반한 경우는 21명(75.0%)이었고, 발달 지연은 27명(96.4%)에서 나타났다. 뇌 MRI 소견으로는 미만성의 피질 위축 소견이 18명(64.3%)에서 보였고, 기저핵의 음영 변화도 12명(42.9%)에서 발견되었다.

결 론 : 사립체 호흡 연쇄 효소 복합체 결함은 간질뿐만 아니라 신경학적 증상을 동반하는 여러 가지 질환에서 원인으로 고려될 수 있으며, 적극적인 진단과 검사가 필요할 것으로 사료된다.

Key Words : Mitochondrial Respiratory Chain Defect, Respiratory chain deficiency, MRC, Epilepsy, Neurological manifestation

Introduction

Mitochondria were first introduced as a cellular microorganism related with human diseases since Luft, et al[1]. proposed evidences of mitochondrial function abnormality in a patient suf-

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fering hypermetabolism in 1962. The role of mitochondria was further evaluated thanks to continuous reports on respiratory chain dysfunction and morphological abnormality in patients with encephalomyopathy since then. In 1963, Nass et al[2]. found out the fact that mitochondria possess their own mitochondrial DNA (mtDNA). In 1981, all the base sequences of mtDNA in both human and mouse were reported[3, 4].

Mitochondrial respiratory chain (MRC) disorders constitute a highly heterogeneous group when observing the clinical manifestations and underlying genetic and biochemical defect[5, 6]. This clinical heterogeneity is due to the dual genetic origin of respiratory chain components such as mtDNA and nuclear DNA[7, 8]. MRC disorders are known to occur with an incidence of 1/10,000 live births and affect mostly organs such as the brain, heart, and skeletal muscle which are highly energy dependent to defects in energy metabolism[9, 10].

Though our understanding of MRC disorders, also known as mitochondrial encephalomyopathies or cytopathies has increased dramatically in recent years, not much is reported concerning clinical manifestations or characteristics in pediatric patients with neurological manifestations. Thus we evaluated 28 children with neurological problems diagnosed as MRC disorder using biochemical assay studies hoping to get one step closer to the way of more precise diagnosis and effective treatments.

Materials and Methods

We included 28 patients with MRC defect among children visited in Severance hospital, Yonsei university college of medicine between 2002 and 2005 suspected to have mitochondrial disorders due to their clinical symptoms including neurologic presentations, progressive course and several screening tests results. Those 28 children were confirmed as suffering MRC defect less than 30% of normal mean data by mitochondrial enzyme activity study.

We retrospectively analyzed the medical records of 28 patients including clinical features and laboratory data such as serum lactate/pyruvate, cerebrospinal fluid (CSF) latate, beta-hydroxybutyric acid/acetic acid ratio and urine organic acid assay.

Muscle biopsy was performed in all patients. Morphologic studies including routine light microscopy, immunohistochemistry, and electron microscopy were performed as well. Mitochondrial enzyme functions were evaluated using methods described by Rustin, et al. analyzing the activities of NADHcoenzyme Q (CoQ) reductase (complex I), succinate-CoQ reductase (complex II), succinate-cytochrome c reductase (complex II, III), cytochrome c reductase (complex III), cytochrome c oxidase (complex IV), oligomycin-sensitive ATPase (complex V), and citrate synthase assessed in isolated mitochondria from muscle tissue using standard spectrophotometric assays[11]. MRC defect was diagnosed when the enzyme activity checked was lower than 2 standard deviation of control mean.

Brain magnetic resonance imaging (MRI) and proton magnetic resonace spectroscopy (MRS) were used for the neuroimaging study in our children.

Patients with symptoms of epilepsy were classified according to clinical semiology and electroencephalography data.

Results

1. Clinical characteristics

Mean age of children was 6.67±4.44 years (range: 1-17.5 years) and their sex ratio was 1.15:1, male to female. As for neurological symptoms, developmental delay, the most common, was reported in 27 cases (96.4%), seizure in 21 cases (75%), motor weakness in 13 cases (46.4%), behavioral change in 4 cases (46.4%), and ophthalmoplegia in 1 case (3.6%) (Table 1). In 15 cases (53.6%), the onset time of clinical symptom was reported as younger that 1 year old, constituting more than half the cases. However, there were 4 cases (14.3%) developed after 5 old years of age as well (Table 2).

Table 1. Neurological Symptoms Associated with MRC Disorders in Children (N=28)

Neurologic symptoms	No. of patients (%)
Developmental delay	27 (96.4)
Seizure	21 (75.0)
Motor weakness	13 (46.4)
Behavior change	4 (14.3)
Ophthalmoplegia	1 (3.6)

Table 2. Onset Age of Clinical Symptoms (N=28)

Onset age	No. of patient (%)
<1 yr.	15 (53.6)
15 yrs.	9 (32.1)
>5 yrs.	4 (14.3)

2. Screening test for mitochondrial disorders

Twenty six (92.9%) among 28 cases showed lactic acidosis while 26 (92.9%) showed plasma lactate/private ratio over 20. Beta-hydroxybutyric acid/acetic acid ratio was checked in 24 patients and 16 (66.7%) children showed ratio over 2.

CSF lactate level was increased in 4 (36.4%) among 11 children who went through the study while lactate excretion was increased 15 (62.5 %) among 24 in urine organic acid assay study (Table 3).

3. Neuroimaging study

In the brain MRI finding of our study group, diffuse cortical atrophy was the most common finding, observed in 18 cases (64.3%). Abnormal signal change of basal ganglia was seen in 12 cases (42.9%), signal change of thalamus in 8 cases (28.6%), and brain stem abnormality in 7 cases (25%) (Table 4).

Definite lactate peak was observed in 11 (57.9%) cases among sthose 19 patients who

Table 3. Sensitivity of Laboratory, Imaging and Pathology Findings in MRC Disorders in Children

Findings	Total No. of patients	Positive finding (%)
Lactic acidosis	28	26 (92.9)
Increased lactate/pyruvate ratio (>20)	28	26 (92.9)
Increase CSF lactate	11	4 (36.4)
Increased betahydroxybutyric acid/acetic acid ratio (>2)	24	16 (66.7)
Abnormal urine organic acid assay	24	15 (62.5)
Lactate peak in MRS	19	11 (57.9)
Abnormal muscle pathology		
Light microscopy	28	6 (26.1)
Immunohistochemistry	28	6 (26.1)
Electon microscopy	28	8 (28.6)

received Proton MRS (Table 3).

4. Biochemical assay for mitochondrial respiratory chain

Regarding types of MRC defect, the most common one was complex I deficiency, observed in 18 cases (64.3%) followed by complex IV deficiency in 8 cases (28.6%) and complex II deficiency in 1 case (3.6%). There was a case showing both type I deficiency and IV (Table 5).

5. Muscle pathology

Ragged red fiber was noted in 6 (26.1%) cases under routine light microscopy including modified Gomori trichrome stain. Abnormal findings were observed in 6 (26.1%) cases in studies of immunohistochemistry including succinate dehydrogenase, NADH, ATPase, PAS, and lipid stain. Under electron microscopy, 8 (28.6%) patients proved to have either abnormal mitochondrial structure including proliferation of the cristae mitochondriales and paracrystalline inclusions, or mitochondrial proliferation with accumulation of excessive or enlarged mitochondria in the subsarcolemmal region (Table 3).

Table 4. Brain MRI Findings Associated with MRC Disorders in Children (N=28)

Findings	No. of patients (%)
Normal	4 (14.3)
Diffuse cortical atrophy	18 (64.3)
Basal ganglia signal abnormality	12 (42.9)
Thalamus signal abnormality	8 (28.6)
Brain stem signal abnormality	7 (25.0)
Cerebellar atrophy	4 (14.3)
Infarction	2 (7.1)
White matter signal abnormality	2 (7.1)

Table 5. Results of Enzyme Deficiency for MRC (N=28)

Enzyme deficiency	No. of patients (%)
MRC complex Ⅰ	18 (64.3)
MRC complex Ⅱ	1 (3.6)
MRC complex Ⅲ	0
MRC complex Ⅳ	8 (28.6)
MRC complex Ⅰ+Ⅳ	1 (3.6)

6. Clinical diagnosis for mitochondrial disorders

As for the clinical diagnosis of mitochondrial disorders of total 28 patients, 8 cases (28.6%) were diagnosed as Leigh syndrome. Though myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS), Kearns-Sayre syndrome, and Alpers syndrome was also diagnosed in one case respectively, it was rather difficult to categorize most of the cases (Table 6).

7. Respiratory chain defect and epilepsy

Twenty one (75.0%) patients were diagnosed as epilepsy. Among them, 7 (33.3%) children showed generalized type and 4 (19.0%) showed partial. 6 (28.6%) cases was diagnosed as Lennox-Gastaut syndrome and 4 (19.0%) cases as infantile spasm (Table 7).

Table 6. Cilinical Diagnosis for MRC Disorders (N=28)

Clinical diagnosis	No. of patients (%)
Leigh syndrome	8 (28.6)
MELAS	1 (3.6)
KearnsSayer syndrome	1 (3.6)
Alpers syndrome	1 (3.6)
Uncategorized	17 (60.7)

Table 7. Classification of Epilepsy Associated with MRC Disorders in Children (N=21)

Epilepsy classification	No. of patients (%)
Infantile spasm	4 (19.0)
LennoxGastaut syndrome	6 (28.6)
Generalized epilepsy	7 (33.3)
Partial epilepsy	4 (19.0)

Discussion

The onset of mitochondrial disorders is known to range from early embryogenesis to late adulthood. Accordingly, mitochondrial disorders can be present at any age[12]. However, in 15 cases, which are more than half of the study group, the onset age was less than 1 year of age pointing out the importance of early observation and detection of related symptoms.

Even though a wide variety of symptoms can be manifested in mitochondria disorders, organs requiring high energy such as brain, muscle, and heart are commonly affected organs causing clinical symptoms or signs[13]. Similar pattern was observed in our study since neurologic symptoms were initial and significant symptoms in all our cases. Seizure and developmental delay were seen quite often while the latter one seen in almost every patient.

Diagnosing mitochondrial disorders is not an easy work because of the genetic heterogeneity, various phenotype and the absence of golden standard[14, 15]. When approaching to a patient with suspected miotochondrial disorders, one has to individualize every case and apply an integral approach, incorporating clinical, electrophysiological, imaging, histological, biochemical and genetic investigations.

As for the screening tests of mitochondrial disoders, increased lactate level, lactate/private ratio implying cytoplasmic redox state and beta-hydroxybutyric acid/acetic acid ratio implying intramitochondrial redox state are considered significant[16, 17]. In our analysis data, lactic acidosis and increased lactate/pyruvate ratio (>20) was checked in 92.3% of patients respectively proving to be useful methods for screening tests. In some cases of encephalomyopathy, lactate was reported to increase in CSF analysis[18, 19]. We also found 4 cases (36.4 %) of increased CSF lactate level out of 11 patients who went through CSF study. But considering its rather low positive test results, CSF lactate study does not seem to be an attractive screening test compared to other methods.

Neuroimaging study does play an important role in diagnosing mitochondrial disorders, but it is also true that it results in nonspecific or normal conclusions in many cases[20, 21]. According to a recent report, mitochondrial disorder associated with clinical central nervous system (CNS) involvement can show deep gray matter signal abnormality and significant lactate peak finding in proton MRS[22]. In our results, diffuse cortical atrophy, basal ganglia signal change, thalamus signal change, and brain stem signal change were observed in the order of high frequency. However, there still were 4 cases (14.3%) with normal findings and wide range of findings such as signal changes of white matter in 2 cases (7.1%). There was no specific correlation between RC complex defect and MRS findings but MRS proved to be a good diagnostic tool showing positive rate of 57.9%.

Biochemical assay on mitochondrial RC enzyme using muscle specimen is a very useful method of examination[14, 23]. Complex I deficiency was found most commonly, in 18 cases (64.3 %), in our patients which correlates other previous reports[24, 25]. But no significant statistic correlation was found between types of complex

deficiency and their clinical features or laboratory findings.

True ragged red fibers are rather rare findings in children with RC defect while subsarcolemmal accumulation of mitochondria (SSAM) is more common[25, 26]. We also found abnormal results more common under electron microscopy than under light microscopy.

Mitochondrial disorders are quite hard to categorize based on clinical diagnosis due to their clinical and laboratory heterogeneity and we had faced the same difficulty as well1[5]. This reflects the fact that diagnosing mitochondrial disorders is a painstaking piece of work. In other words, it also implies that we should always consider the possibility of mitochondrial disorder in patients with progressive clinical course and variable clinical or laboratory findings. Since there are often many cases hard to divide into certain mitochondrial syndrome even when evidences of MRC defect are present, we should pay a close and continuous attention trying to characterize the syndrome with specific categories.

Epileptic seizures are the presenting sign of many mitochondrial disorders with CNS involved. Mitochondrial dysfunction can be suspected to be an important cause of epileptic seizures and therapy-resistant forms of severe epilepsy, since impairment of mitochondrial function is observed in seizure focus of human brain[27, 28]. Many different types of mutations of mitochondrial DNA leading to selective inhibition of mitochondrial oxidative phosphorylation in epileptogenic areas of the human brain has been related with epileptic phenotypes[29]. Not much has been reported on the issue of epileptic phenotype. 21 cases (75.0%) of our children suffered epilepsy not only including generalized epilepsy and partial epilepsy, but also epileptic syndrome such as infantile spasm and Lennox-Gastaut syndrome. This reassures the fact that though no certain relationship is established between types of respiratory chain defect and epileptic phenotype, respiratory chain defect is certain to be a cause of epilepsy.

Mitochondrial cocktail therapy including l-carnitine, coenzyme Q10, and high doses of multivitamins was introduced as a treatment modality in mitochondrial disorder[30]. 75% of patients seemed to experience improvement after the therapy based on subjective judgments of the caretakers, but more objective study and evaluation is recommended.

In short, though mitochondrial disorders are multisystem disorders and not easy to diagnose, MRC defect should be considered as an important cause of idiopathic neurological disorder including epilepsy. Due to lack of a established causative treatment, ketogenic diet and mitochondrial cocktail therapy are now provided. Recent studies are reported discussing their possibilities as an effective way of therapy, more extensive studies on these modalities will be helpful in treating mitochondrial disorders in the future.

Abstract

Purpose : The study was carried out to characterized the clinical and the laboratorial features of children with mitochondrial respiratoy chain disorders in Korea.

Methods : We retrospectively analyzed the clinical and the loboratorial data of 28 children with significantly low activities in respiratory chain complexes of muscle using spectrophotometry.

Results : The mean age was 6.67±4.44 years and the ratio males to female was 1.15:1. Eighteen patients (64.3%) showed defects in Complex I, 8 (28.6%) in Complex VI, 1 (3.6%) in Complex II, and 1 in Complex I and IV. Eight cases (28.6%) were diagnosed with Leigh disease, one with MELAS, Kearns-Sayre syndrome, and Alpers disease retrospectively, but the predominant clinical presentations were a non-specific encephalopathy (17/28, 60.7%). Epilepsy was seen in 21 (75.0%) patients, while developmental delay in 27 (96.4%) patients. Fifteen out of 28 children (53.6%), clinical symptoms mostly appeared below age of 1 year. The brain MRI showed diffuse cortical atrophy in 18 (64.3%) patients and basal ganglia signal changes in 12 (42.9%) patients.

Conclusion : The defects in mitochondrial respiratory chain complexes should be considered in any children with an unexplained neurological condition including even epilepsy.

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