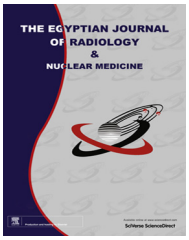
The Egyptian Journal of Radiology and Nuclear Medicine (2014) **45**, 523–533

Egyptian Society of Radiology and Nuclear Medicine

The Egyptian Journal of Radiology and Nuclear Medicine

www.elsevier.com/locate/ejrnm
www.sciencedirect.com



ORIGINAL ARTICLE

Role of brain magnetic resonance spectroscopy in the evaluation of suspected mitochondrial diseases in children: Experience in 30 pediatric cases

Ahmed A. ElBeheiry [a,*], Ahmed M. Abougabal [a], Tarek I. Omar [b], Ashraf N. Etaby [a]

[a] *Department of Radiodiagnosis, Faculty of Medicine, University of Alexandria, Egypt*
[b] *Department of Pediatrics, Faculty of Medicine, University of Alexandria, Egypt*

Received 9 November 2013; accepted 30 December 2013
Available online 31 January 2014

KEYWORDS

Magnetic resonance spectroscopy;
Mitochondrial diseases;
Lactate

Abstract *Introduction:* Mitochondrial diseases are a group of inherited disorders caused by derangement of mitochondrial respiration. MR spectroscopy (MRS) has been shown to detect abnormal accumulation of lactate in brain parenchyma and CSF in patients with mitochondrial disorders, but the frequency of detection is largely unknown.
Aim of the work: To evaluate the role of brain MR spectroscopy in the assessment of suspected mitochondrial diseases in the pediatric age group.
Patients and methods: Thirty children with suspected mitochondrial diseases were examined by MRS. Examination was done using multisection technique and multiple echo times mainly short (35 ms) and intermediate (144 ms). Mitochondrial disease criteria scoring system was used to confirm the suspected diagnosis.
Results: All patients showed elevated lactate peak with the CSF being the most sensitive (100%). Among the 30 patients, 26 (86.7%) had elevated levels of blood lactate/pyruvate ratio. Conventional MRI showed highly suggestive features in 15 patients while non specific findings were detected in 11 patients and 4 showed normal appearing brain.
Conclusions: MRS provides a noninvasive tool for the diagnosis of mitochondrial diseases, especially in children with non specific findings on MRI, normal appearing MRI or a normal blood lactate/pyruvate ratio.

* Corresponding author. Tel.: +20 1223926478.
E-mail address: elbeheiryahmed@gmail.com (A.A. ElBeheiry).
Peer review under responsibility of Egyptian Society of Radiology and Nuclear Medicine.

ELSEVIER Production and hosting by Elsevier

0378-603X © 2014 Production and hosting by Elsevier B.V. on behalf of Egyptian Society of Radiology and Nuclear Medicine.
Open access under CC BY-NC-ND license. http://dx.doi.org/10.1016/j.ejrnm.2013.12.012

1. Introduction

Mitochondrial disorders (MDs) are no longer regarded as rare disorders, and approximately one-third of inherited metabolic disorders in children are attributable to mitochondrial defects (1). Mitochondrial disorders (MDs) are a family of genetically and clinically heterogeneous diseases characterized by defective energy production. Their heterogeneity is due in part to the biochemical complexity of mitochondrial respiration and the fact that two genomes, one mitochondrial and the other nuclear, encode the subunits of the respiratory chain complexes (2).

Mitochondrial disorders (MDs) manifest in tissues and organs with high-energy demands, with the central nervous system (CNS) being the second most frequently affected system after the muscular system because of its strong dependence on oxidative phosphorylation (3). CNS manifestations are found in both syndromic and non-syndromic mitochondrial disorders with the most common known mitochondrial syndromes include Leigh syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke like episodes (MELAS), Kearns–Sayre syndrome (KSS), and Alpers disease (4).

Clinical manifestations of MDs in the pediatric age group are often nonspecific because symptoms may arise from any organ or system. Although recent advances in genetics have led to the development of diagnostic tools for many MDs, still most of the patients present with challenging diagnostic dilemmas. Therefore, diagnostic confirmation of a MD is based on a combination of modalities including clinical features, metabolic survey, neuroradiologic findings, histopathologic analysis of muscle specimens, and molecular genetic studies (4).

Apart from the clinical, biochemical and morphological investigations, neuroimaging is of great importance when diagnosing mitochondrial disorders with CNS involvement. Since the study by Barkovich et al. (5), claims that the combination of deep gray matter and peripheral white matter involvement in children should suggest a mitochondrial disorder, the field of neuroimaging has witnessed a marked progress. Both structural MRI and functional brain imaging methods, including magnetic resonance spectroscopy (MRS), diffusion-weighted imaging (DWI) and perfusion MRI, have helped greatly to increase our knowledge of mitochondrial disorders by allowing a non-invasive pathological assessment of the anatomic lesions, metabolism and hemodynamics of the brain (6).

MRI signal abnormalities, can reveal specific or 'signature' disease features (as in MELAS syndrome), non-specific features or leukodystrophic-like features suggesting a mitochondrial disorder, while a structurally normal brain on MRI may also be found (7,8).

The most common known radiological features of mitochondrial syndromes include bilateral symmetrical signal abnormalities, most frequently in the lentiform (putamen more often than globus pallidus) and caudate nuclei, as well as periaqueductal gray matter, red nuclei and dentate nuclei in Leigh syndrome, stroke-like lesions which are often transient with the lesions predominantly affecting the occipital gray matter, yet not confined to vascular territories in MELAS; and the involvement of the subcortical U fibers with sparing of the periventricular white matter in Kearns–Sayre syndrome (KSS) which differentiates it from most lysosomal and peroxisomal disorders where subcortical regions are only affected late in the disease (9).

MRS is an MR-based, non-invasive method to get chemical information from the brain. The development of proton MRS (H-MRS) has made it possible to study both normal and abnormal metabolite concentrations in the pediatric brain at different developmental stages. These include N-acetyl aspartate (NAA), creatine (Cr), choline (Cho), myoinositol (myo-I), glutamate, glutamine, and lactate (10).

When oxidative phosphorylation is impaired as in mitochondrial diseases, energy metabolism follows the alternative route of anaerobic glycolysis and produces lactic acid. Lactate has a chemical shift of 1.33 ppm and presents as a doublet peak on in vivo H-MRS due to coupling effects (10). Lactate elevation has been found as the most prominent MRS signal abnormality in mitochondrial diseases (6).

A consensus mitochondrial disease criteria scoring system (MDC) has been recently established to facilitate the diagnostic work-up of patients with suspected mitochondrial disorders (11). MDC is based on a multidisciplinary approach that includes clinical signs and symptoms, metabolic/imaging studies and muscle histology. Among these criteria brain magnetic resonance imaging (MRI) and brain magnetic resonance spectroscopy (MRS) examinations play an important role, especially in children, since MR findings may influence the decision to precede with additional specific tests such as molecular analysis (which is not available at every center) or muscle biopsy (which is an invasive procedure) (8,11).

The aim of this study was to evaluate the role of brain MRS in the assessment of suspected mitochondrial diseases in the pediatric age group.

2. Materials and methods

Thirty children with clinically suspected diagnosis of mitochondrial disease were referred for MR imaging and MR spectroscopy between October 2011 and May 2013. Detailed neuro logical examination and basic laboratory tests were performed on all patients. The laboratory tests consisted of analysis of arterial blood gas, blood lactate level (normal 3–12 mg/dl), lactate/pyruvate ratio (normal less than 18) and metabolic surveys including assays of blood amino acids and urinary organic acids, with lactate/pyruvate ratio being the main laboratory test which we depended upon in our analysis.

2.1. MRI imaging

All patients in our study population required sedation or anesthesia. Pre-scanning preparation included 3–6 h fasting. MR imaging and MR spectroscopy examinations were performed in a single session in most cases with a 1.5-T MR unit (Signa Horizon 1.5; GE Medical Systems, Milwaukee, WI). T1-weighted images [echo time (TE)/repetition time (TR) 11 ms/550 ms], T2-weighted images (TE/TR 93 ms/4000 ms), fluid-attenuated inversion recovery (FLAIR) (TE/TR/inversion time 110 ms/10000 ms/2250 ms) and diffusion weighted images (DWI) (TE/TR: 105 ms/5200 ms) were performed.

Axial and sagittal T1-weighted images were assessed in particular for the presence of cortical atrophy, agenesis/hypoplasia of the corpus callosum, enlargement/dilatation of the ventricles and/or subarachnoid spaces. Axial and coronal T2-weighted images, including fluid attenuated inversion recovery (FLAIR) images, were studied to identify signal intensity

Table 1 Distribution of the studied cases according to different parameters.

	Number	%
Age		
< 1 year	9	30.0
1 – 2 years	13	43.3
> 2 years	8	26.7
Sex		
Male	16	53.3
Female	14	46.7
L/P ratio		
Normal	4	13.3
Elevated	26	86.7
MRI findings		
Highly Suggestive	15	50
Non specific	11	36.6
Normal	4	13.3
MRS + ve lactate		
MRS + ve Basal ganglia lactate	29	96.7
MRS + ve White matter lactate	19	63.3
MRS + ve CSF lactate	30	100

changes mainly within the basal ganglia and cortex. Myelination was assessed on T1 sequences in children younger than 6 months, T1 and T2 sequences in the age group between 6 months and 1 year and on T2 in children older than 1 year. Myelination was compared to age-matched healthy controls. DWI was used to assess the presence of cytotoxic or myelin edema both showing restricted diffusion.

According to pattern specific approach adopted by Barkovich, (12) MRI findings were categorized on the basis of their predominant location in the brain as follows: lesion predominance in the basal ganglia; cerebral cortex; white matter; combined gray and white matter; normal MRI features (no definite abnormality on conventional MRI basis).

2.2. MR spectroscopy

MR spectra were obtained by using chemical shift imaging (CSI) with application of a multisection spectroscopic imaging technique (15-mm section thickness) spin-echo sequence (2300/280/1) with outer volume suppression. The field of view was 24 cm, with 32×32 phase-encoding steps, rendering a nominal voxel size of 0.8 cm^3. Water suppression was accomplished with a single chemical shift selective pulse with a bandwidth of 110 Hz. Extracranial lipid signals were attenuated by the use of eight outer volume saturation pulses. The sections were chosen so that the most caudal section was at the level of the third ventricle, slightly above the tentorium; the second section included the lateral ventricles and basal ganglia; the third section was at the level of the centrum semiovale; and the top section contained mainly cortical gray matter.

Voxels were sampled using multiple echo times, including 35 ms (short), and 144 ms (intermediate) with repetition times of 2000 ms.

A volume of interest for MRS was positioned on the axial MRI with one or multiple regions within the basal ganglia (whether normal or abnormal), another within the white matter (whether normal or abnormal) and the last within the ventricular CSF. All spectra were reviewed with a special interest in lactate level elevation. A peak at 1.33 ppm at a TE of

Table 2 Distribution of the studied cases as regards MRS and scoring results according to different MRI groups.

Group	Number of patients	Percentage	Scoring before MRS	Final score after MRS	Number of patients upgraded after MRS
Group IA (13 patients)...Leigh					
+ ve BG lactate	13	100	10 probable	2 definite	
+ ve WM lactate	7	53.8	3 possible	11 probable	
+ ve CSF lactate	13	100			
Group IB(1 patient)...MELAS					
+ ve BG lactate	1	100	1 possible	1 probable	6 out of 15 patients (40%)
+ ve WM lactate	1	100			
+ ve CSF lactate	1	100			
Group IC (1 patient)...Kearns–Sayre					
+ ve BG lactate	1	100	1 probable	1 probable	
+ ve WM lactate	1	100			
+ ve CSF lactate	1	100			
Group IIA(5 patients)...cortical atrophy					11 out of 11 patients (100%)
+ ve BG lactate	5	100	5 possible	5 probable	
+ ve WM lactate	2	40			
+ ve CSF lactate	5	100			
Group IIB (6 patients)...combined gray and white matter					
+ ve BG lactate	6	100	6 possible	6 probable	
+ ve WM lactate	6	100			
+ ve CSF lactate	6	100			
Group III (4 patients)...Normal MRI					
+ ve BG lactate	3	75	4 possible	3 probable	3 out of 4 patients (75%)
+ ve WM lactate	2	50		1 possible	
+ ve CSF lactate	4	100			

35 ms, or an inverted doublet at a TE of 144 ms, was used to diagnose the acquisition of lactate.

We analyzed the MRS findings in children with suspected mitochondrial diseases, which were then compared with the results of MRI done in the same setting and other diagnostic non radiological methods including blood lactate/pyruvate ratio.

2.3. Scoring system

We used the consensus mitochondrial disease criteria (MDC) scoring system, (11) which evaluates clinical manifestations (I with maximum 4 points), metabolic and imaging studies (II with another 4 points as maximum), and morphologic or histopathologic criteria (III with maximum 4 points) with the total score of 12. The clinical manifestations are subdivided into 3 main groups; IA: muscle symptoms including ophthalmoplegia, exercise intolerance and muscle weakness with maximum 2 points, IB: CNS abnormalities including developmental delay, seizures, pyramidal and extrapyramidal signs, also with maximum of 2 points and lastly IC: multisystem disease including affection of extra CNS systems as heart, kidney, vision and hearing with maximum of 3 points. The metabolic and imaging studies (class II) include laboratory abnormalities as elevated serum lactate, lactate/pyruvate ratio and alanine along with imaging abnormalities including Leigh like features (sharing with 2 points), stroke like

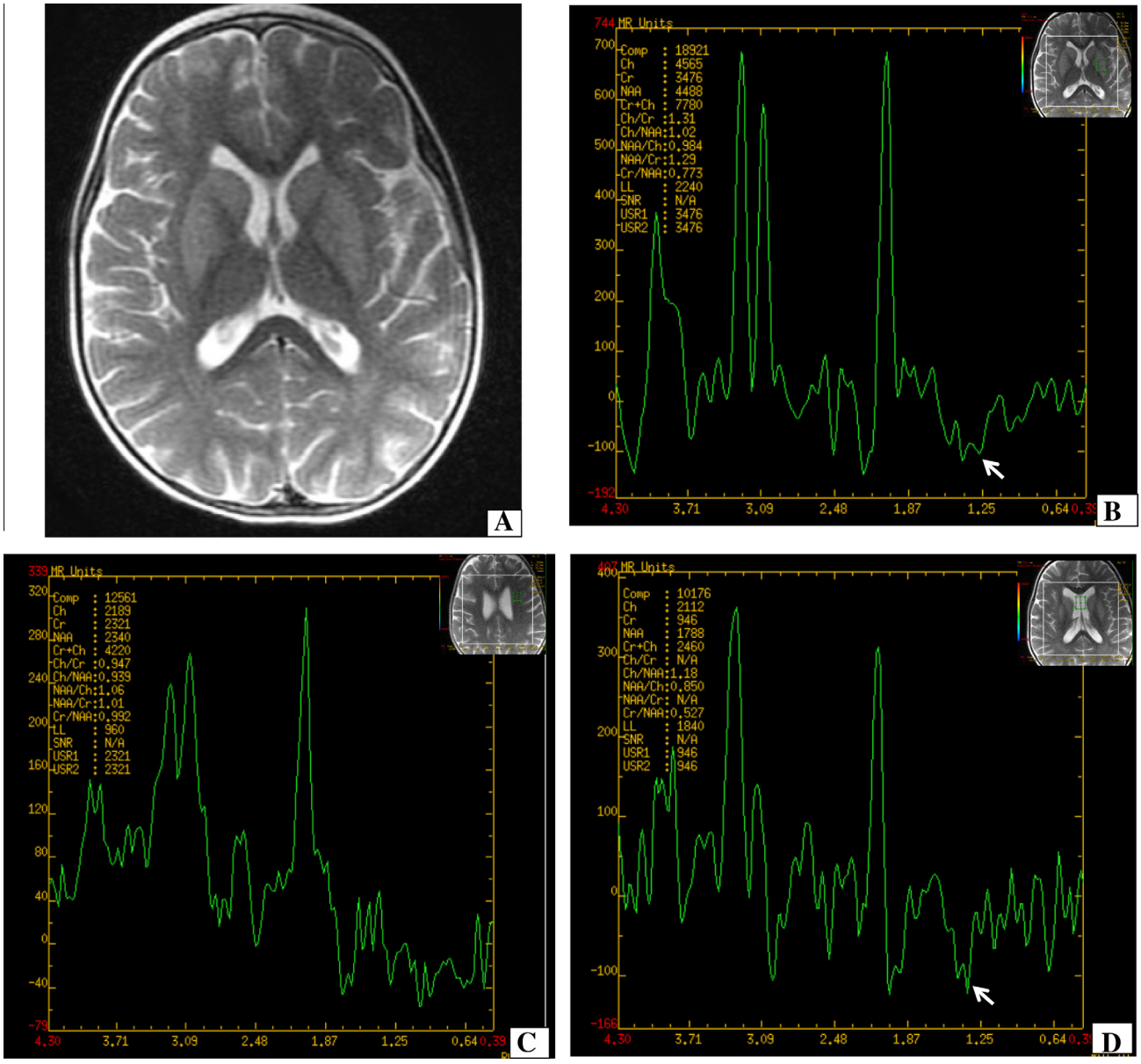


Fig. 1 2 year old male presenting with extrapyramidal manifestations associated with seizures with normal L/P ratio. MRI features suggestive of Leigh disease: (A) axial T2 showing bilateral symmetrical hyperintense signal of the caudate, putamina and globus pallidus with preserved white matter. (B–D) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the basal ganglia and CSF (white arrows in B and D) with no lactate within the white matter corona radiata (C). This child had pre MRS score of 4 (possible MD) which has changed into 5 (probable MD) after MRS.

manifestations, cortical or cerebellar atrophy, and leukodystrophy (each sharing with only one point) as well as abnormal brain MRS in the form of elevated lactate (also sharing with one point). Maximum of 4 points are collected from this class. Histologic abnormalities (III) including abnormal mitochondria on electron microscopy could increase the score with 4 points, leading to a maximal score of 12 points (11). A score of 1 makes mitochondrial disease unlikely, from 2 to 4 makes it possible, from 5 to 7 is comparable with a "probable mitochondrial disorder"; while score of 8–12 confirms the diagnosis ("definite mitochondrial disorder"). Yet in our study and due to the absence of histological assessment, the final score was out of 8 (4 from the clinical score and 4 from the laboratory and neuroimaging score).

3. Results

30 patients ranging from 2 months up to 10 years of age were included in this study presenting with wide spectrum of clinical manifestations which were suspected to be sequel to mitochondrial disease. Among the 30 patients, 26 (86.7%) had corresponding elevated levels of blood lactate/pyruvate ratio, and four (13.3%) had normal levels. (Table 1).

Conventional MRI showed three main groups: group (I) with highly suggestive features included 13 patients with Leigh like features, one with MELAS appearance and one with Kearns Sayre disease like findings (total of 15 patients mounting to 50%), group (II) with non specific features included 11 patients (36.6%); 5 of which with cortical atrophy and 6 with combined gray and white matter affection while the last group (III) included 4 patients (13.3%) with normal appearing brain (Table 1).

All 30 patients showed elevated lactate peak on MRS with variable degrees according to the site of detection with the CSF being the most sensitive (100%), followed by the basal ganglia whether affected or not on MRI(96.7%) and lastly the white matter whether involved or not on conventional MRI (63.3%) (Table 1).

Consequently MRS has upgraded the score of considerable number of patients (20 out of the 30 examined children mounting to 66.6%) with the most considerable effect being noticed in groups II and III; i.e. those with non specific or normal findings on MRI (Table 2).

3.1. Classification of patients according to MRI features

3.1.1. Group I (patients with highly suggestive features)

This group included 15 patients, 13 of which with Leigh like features (group IA), one with MELAS features (group IB) and one with Kearns–Sayre like picture (group IC).

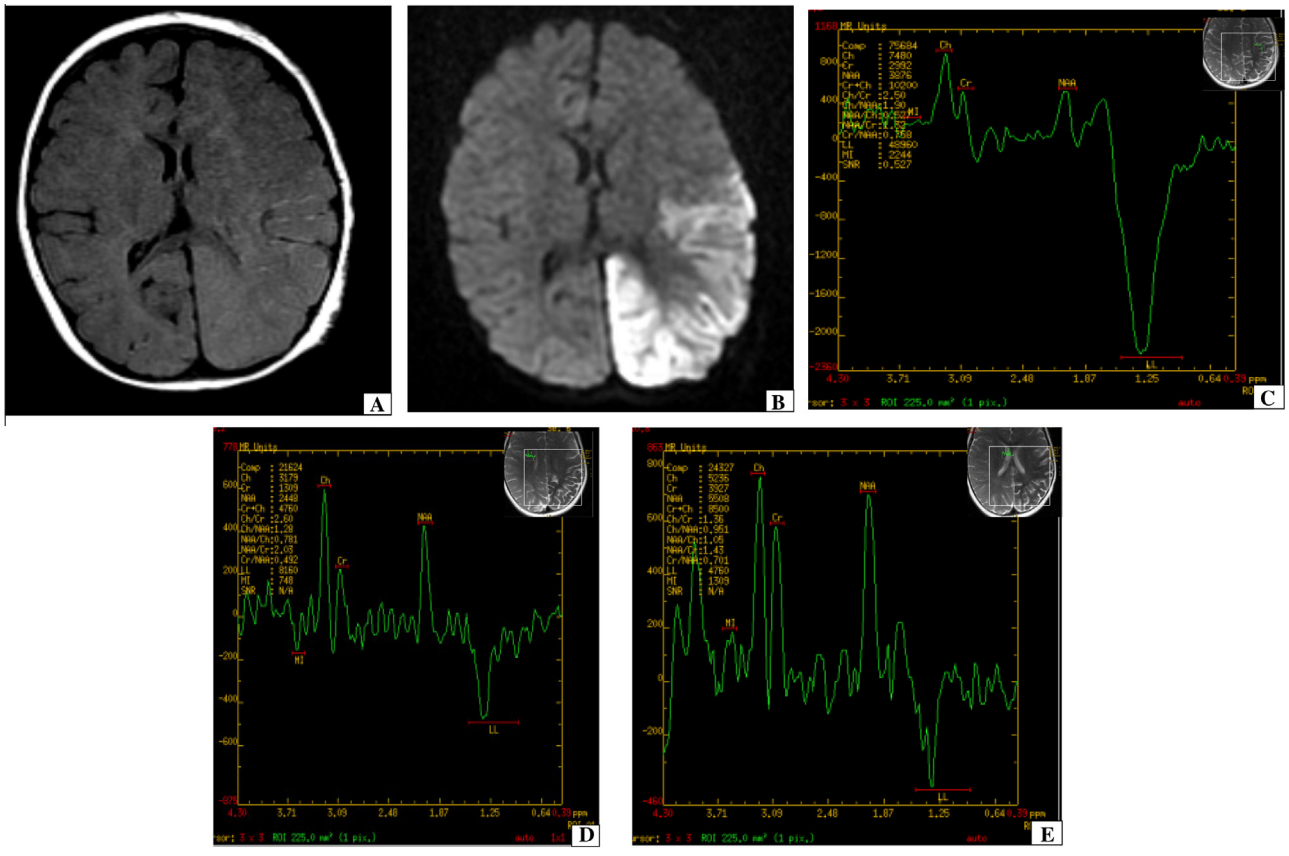


Fig. 2 1 year old female presenting with right hemiparesis and exercise intolerance with abnormally elevated L/P ratio. MRI features suggestive of MELAS: (A) axial T2 FLAIR showing no definite abnormal signal, but tumefactive cortical thickening on the left side with mass effect on the left lateral ventricle. (B) DWI showing restricted diffusion along the left occipito-parietal hemisphere. (C–E) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the affected hemisphere, normal appearing contralateral side, and CSF (C–E), respectively. This child had pre MRS score of 4 (possible MD) which has changed into 5 (probable MD) after MRS.

3.1.1.1. Group IA: patients with features of Leigh disease (bilateral symmetrical basal ganglia involvement): (Fig. 1). 13 patients (5 males and 8 females) were included in this group, most of which presented clinically with extrapyramidal manifestations and 10 had elevated Lactate/pyruvate ratio while 3 showed normal ratio. The highest percentage of this group (7 patients) showed bilateral symmetrical involvement of caudate and putamen followed by bilateral globus pallidus (GP) isolated affection (3 patients), with 2 patients showing involvement of the entire basal ganglia and one with isolated putaminal involvement. MRS showed elevated lactate peak (in the form of inverted doublet at 1.33 ppm on intermediate TE) in the basal ganglia and CSF of all patients (100%) while only 7 patients (53.8%) showed detectable lactate within the normal appearing white matter. 10 patients were categorized as probable and 3 as possible according to the scoring system prior to MRS. Final post MRS scoring changed the categories of two patients from probable to definite (8 points instead of 7), three patients from possible to probable (5 points instead of 4) while the rest were upgraded into higher probability(from 6 to 7 and from 5 to 6 points). (Table 2).

3.1.1.2. Group IB: patient with features of MELAS (stroke like lesions not confined to vascular territory): (Fig. 2). One female patient was included in this group, presenting with acute onset of right hemiparesis along with exercise intolerance and elevated Lactate/pyruvate ratio. MRI showed abnormal signal involving the cortical and subcortical regions of the left parieto-occipital cerebral hemisphere along non vascular territory mainly evident on diffusion weighted images. MRS in such case showed detectable high lactate within the affected region together with the normal appearing basal ganglia, white matter as well as CSF. Pre MRS scoring was 4 (possible MD) which has upgraded into 5 (probable MD) after MRS. (Table 2).

3.1.1.3. Group IC: patient with features of Kearns–Sayre disease (isolated subcortical and peripheral white matter affection sparing the periventricular white matter): (Fig. 3). One male patient was included in this group presenting with global developmental delay (GDD) and seizures combined with external ophthalmoplegia. Lactate/pyruvate ratio was elevated. MRI showed subcortical and deep peripheral white matter

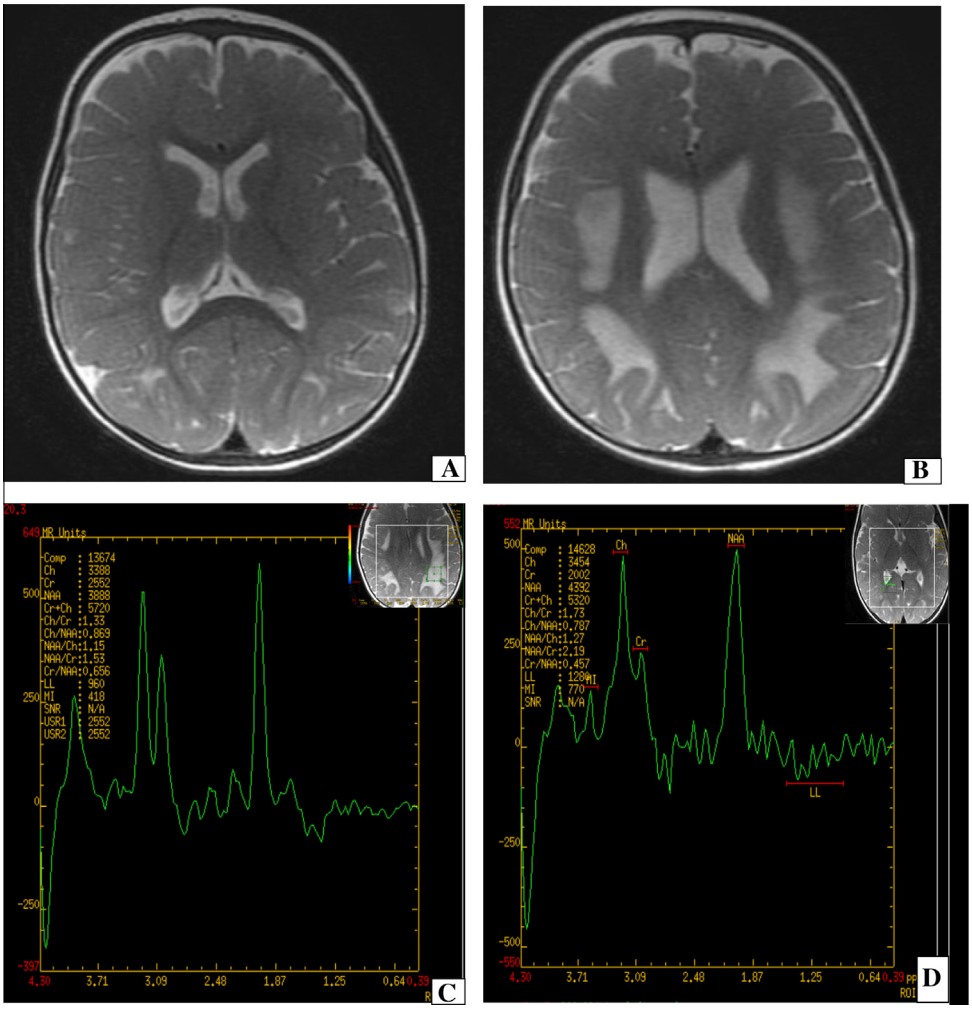


Fig. 3 1 year old male presenting with external ophthalmoplegia combined with developmental delay and seizures along with elevated L/P ratio. MRI features are suggestive of Kearns–Sayre Syndrome. (A and B) Axial T2 images showing normal basal ganglia with abnormal T2 hyperintensity involving the subcortical and deep white matter sparing the periventricular white matter. (C and D) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the white matter and CSF, respectively. This child had pre MRS score of 6 (probable MD) which has changed into 7 (still probable MD) after MRS.

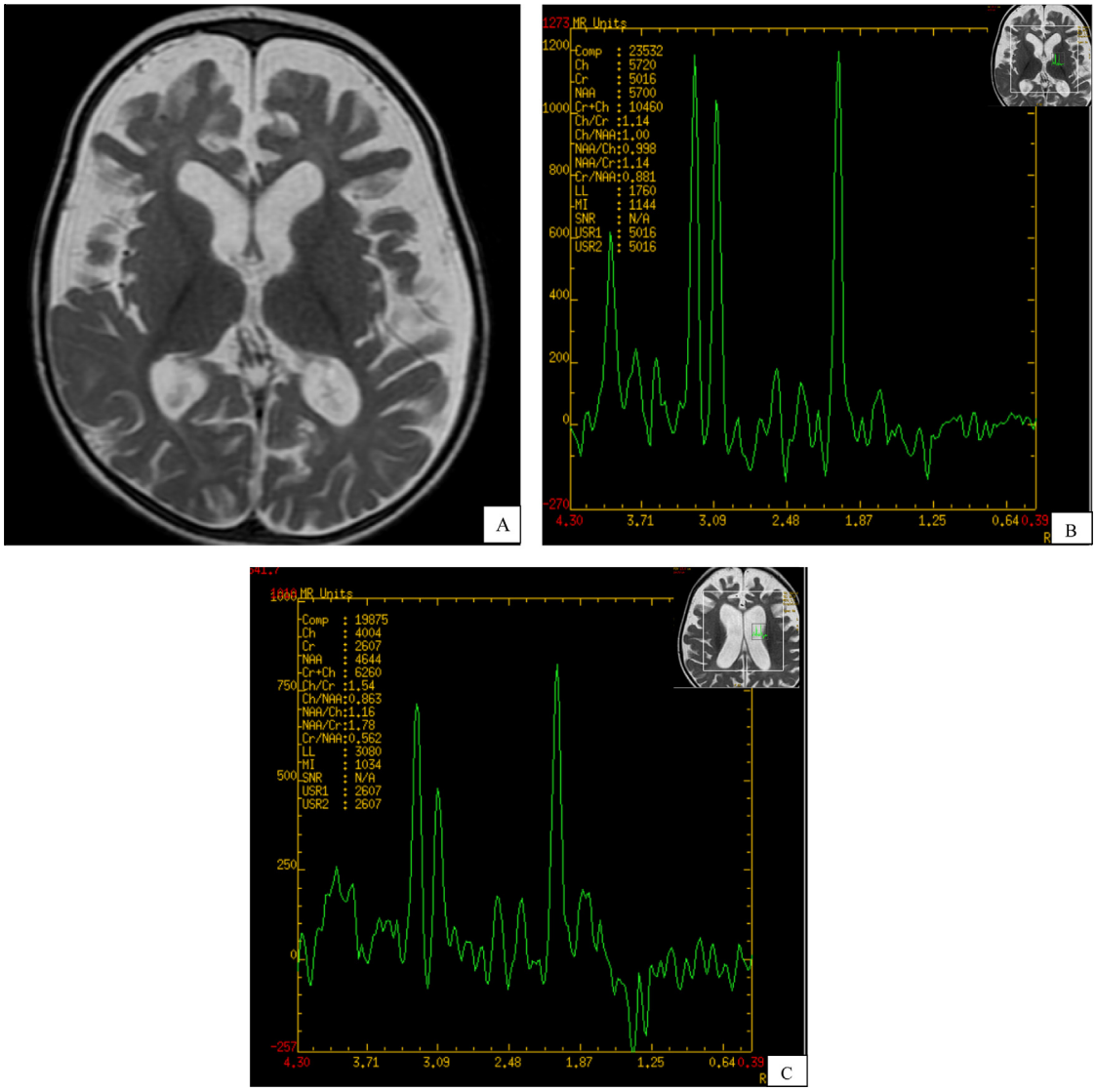


Fig. 4 2 year old male presenting with global developmental delay and seizures with elevated L/P ratio and non-specific MRI features: (A) axial T2 showing diffuse cortical atrophy with preserved basal ganglia. (B and C) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the normal appearing basal ganglia and CSF (B and C), respectively. This child had pre MRS score of 4 (possible MD) which has changed into 5 (probable MD) after MRS.

affection sparing the periventricular white matter with normal basal ganglia. MRS showed elevated lactate peak in the normal appearing basal ganglia and CSF along with the affected white matter. Scoring prior to MRS was 6 points matching with probable and changed into 7 points after MRS, still within the probable range (Table 2).

3.1.2. Group II (patients with non-specific MRI features)

11 patients were included in this group, 5 of which showed cortical atrophy (group IIA) and the other 6 showed combined gray (cortical or basal ganglia) and white matter affection.

3.1.2.1. Group IIA: patients with cortical atrophy and normal basal ganglia: (Fig. 4). 5 patients (3 males and 2 females) were included in this group, presenting with combination of seizures and pyramidal signs. Lactate/pyruvate ratio was elevated in 4 patients and normal in one. MRI showed spectrum of subnormal development to frank atrophic changes manifested as widening of cortical sulci mainly fronto-temporal with deepening of the gyri and basal cisterns along with supratentorial ventricular dilatation. MRS of these 5 patients showed elevated lactate peak in the normal appearing basal ganglia and CSF of all patients while only 2 patients showed detectable lactate within the normal appearing white matter. All patients were upgraded from possible to probable according to scoring after MRS (5 points instead of 4) (Table 2).

3.1.2.2. Group IIB: patients with combined gray and white matter affection: (Fig. 5). 6 male patients were included in this group, 5 of which presented with global developmental delay (GDD) and one with post infectious psychomotor regression.

Lactate/pyruvate ratio was elevated in all patients. MRI showed subnormal development to frank atrophy with dysmyelination in 2 patients, frank atrophic changes along with cystic white matter in another 2 patients with one showing combination of atrophy, cystic white matter, basal ganglia and brain stem affection and the last showing frank atrophy with white matter and brain stem involvement sparing the basal ganglia. MRS showed elevated lactate peak in the basal ganglia, affected white matter and CSF of all patients. All patients were categorized as possible (4 points) prior to MRS and changed into probable (5 points) after MRS. (Table 2).

3.1.3. Group III: patients with normal appearing brain on MRI: (Fig. 6)

4 patients (3 females and one male) were included in this group, 3 of which presented with GDD and pyramidal signs and one with neonatal onset seizures. Lactate/pyruvate ratio was elevated in all patients. MRI was completely normal for age. MRS showed elevated lactate peak (in the form of inverted doublet at 1.33 ppm on intermediate TE) in the CSF of all patients while 3 patients showed detectable lactate peak within the normal appearing basal ganglia and two within the

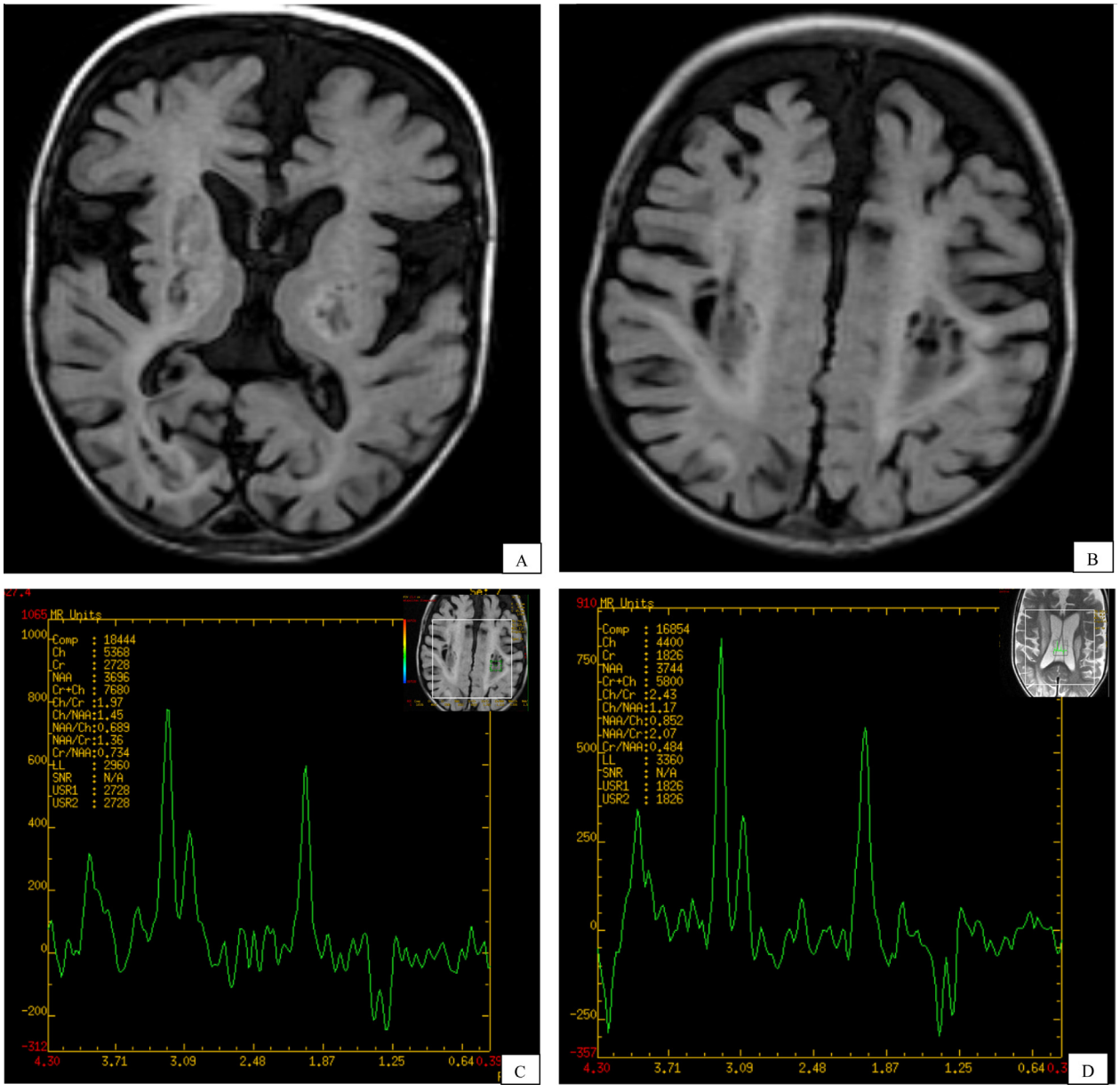


Fig. 5 2 year old male presenting with pyramidal signs along with developmental delay and elevated L/P ratio. MRI non-specific features include; (A and B) axial T2 FLAIR showing diffuse cortical atrophy with bilateral symmetrical white matter hyperintensity showing cystic changes with similar changes noted within the basal ganglia bilaterally. (C and D) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the white matter and CSF, respectively. This child had pre MRS score of 4 (possible MD) which has changed into 5 (probable MD) after MRS.

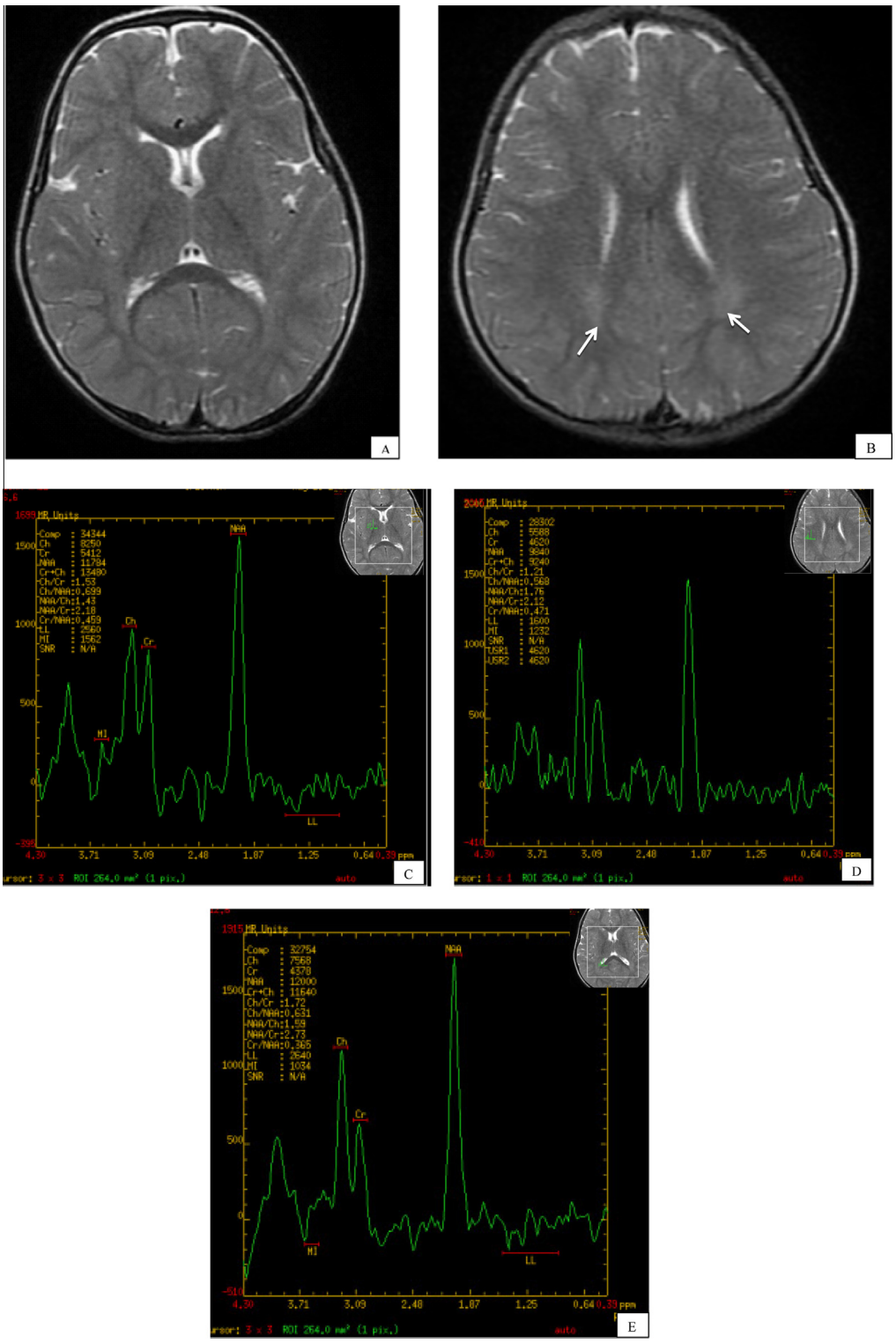


Fig. 6 2 year old female presenting with global developmental delay and elevated L/P ratio. (A and B), Axial T2 showing normal basal ganglia and white matter for age. Note terminal zones of myelination at the peritrigonal regions (arrows at B). (C–E) MRS with intermediate TE showing detectable lactate inverted doublet at 1.33 ppm within the basal ganglia, and CSF (C and E) with no detection within the normal appearing white matter (D). This child had pre MRS score of 4 (possible MD) which has changed into 5 (probable MD) after MRS.

normal appearing white matter. Three patients were upgraded from possible to probable score (5 instead of 4 points) after MRS while one remained within the possible range (4 instead of 3 points) (Table 2).

4. Discussion

Diagnosis in infants and children with mitochondrial diseases is a challenge because of the lack of specific clinical characteristics, sensitive biochemical markers, and a single algorithm (13). The CNS is frequently involved in mitochondrial diseases because of its strong dependence on oxidative metabolism. The clinical consequences of this involvement are extremely heterogeneous, and in only a few cases does the coincidence of particular symptoms and signs depict distinct mitochondrial syndromes as stroke like manifestations in MELAS, and external ophthalmoplegia combined with neurological manifestations in Kearns–Sayre disease (13,14).

Due to its high resolution and absence of ionizing radiation, MRI is considered the corner stone in imaging of inherited neurodegenerative disorders including suspected mitochondrial diseases, yet due to its wide range of findings, MRI alone cannot be the sole imaging modality for definite diagnosis in such conditions. In our investigation 15 out of 30 patients mounting to 50% presented with highly suggestive findings, 13 of which with Leigh like features, one with MELAS and one with Kearns Sayre disease. Yet, similar percentage (50%) presented with non specific findings ranging from cortical atrophy to leukodystrophy like picture (11 patients mounting to 36.6%) with even 4 patients (13.3%) showing normal MRI findings.

Our results are in agreement with the study of Kim et al. (1) who investigated the role of MRI in 40 children with mitochondrial diseases and found 19 to have known syndromes, 19 with non specific findings and 2 with normal MRI features. It is also matching with a more recent study done by Sofou et al. (6) who studied 66 children having CNS manifestations of mitochondrial diseases and shown that 8 patients had normal MRI examinations with more than 50% of the cases with non specific findings. This is supporting the hypothesis that mitochondrial brain diseases show non specific MRI features in considerable number of patients which may lead to false interpretation.

In mitochondrial disease, disruption of the respiratory chain and consequent depletion of NAD+ and NADP+ shift the predominant catabolic metabolism from the Krebs cycle to anaerobic glycolysis. This in turn produces an accumulation of pyruvate and its reduced product, lactate (15). In patients who manifest characteristics suggesting disruption of mitochondrial respiration and in whom hypoxia and increased CNS demand for adenosine 5′-triphosphate (as with active seizures) can be excluded, an elevated lactate can be evidence that confirms the presence of a mitochondrial disorder (15).

Clinically, elevated serum lactate level and lactate/pyruvate (L/P) ratio is only a fair marker of mitochondrial disease (15). The serum lactate detection presents low sensitivity in the diagnosis of mitochondriopathies because it is transported to the liver where it is normally re-oxidized into pyruvate. In normal persons, CSF lactate levels are lower than serum levels, (15) and this metabolite is not detected by routine MRS neither in brain parenchyma nor in CSF. Therefore, the quantification of this metabolite in the CSF provides important information about the presence of anaerobic metabolism in encephalic tissues (4).

In their analysis of 49 children with highly suspected mitochondrial disorder, Dinopolous et al. (16) found that serum lactate concentration was normal in 7 of 49 patients, including three children with Leigh syndrome. Our study agrees with Dinopolous et al. (16) that normal levels of serum lactate and L/P ratios cannot rule out mitochondrial diseases, as in our study lactate/pyruvate ratio was normal in 4 patients (13.3%), 3 of which had MRI features highly suggestive of Leigh disease.

In another study, Nissenkorn et al. (17) have demonstrated similar sensitivity between the CSF lactate detection by MRS and other biochemical, genetic, and histopathological markers of these diseases. This is matching with our results which showed that MRS lactate in CSF to be the most sensitive marker in detection of suspected mitochondrial diseases as lactate was detected in CSF of all patients (100%) including those with normal levels of serum L/P ratio.

Some authors have demonstrated a higher sensitivity of MRS compared to conventional MR scans in the detection of abnormalities in symptomatic patients and individuals at risk for mitochondrial disease, who still have not developed neurological deficit (15,16). The frequency of detectable elevation of lactate on MRS in cases with syndromic MDs or definite MDs reported in the literature ranges from 62.5% (5 of 8) (16) to 81.3% (13 of 16) (15). In our case series, all patients showed elevated lactate peak with difference in degree of acquisition according to the site of examination ranging from 100% in CSF to 63.3% in white matter (whether affected or not) with basal ganglia detection reaching 96.7%.

Sensitivity of brain MRS in mitochondrial diseases in our study was best detected in 4 patients (group III) who had normal appearing brain on conventional MRI but showed detectable lactate peak on MRS which was the sole imaging clue to guide the route for diagnosing mitochondrial disease. This was fortified by applying the scoring system where 3 out of the 4 patients were upgraded from possible into probable grade.

Our study is one of the few studies measuring lactate in normal appearing brain, yet showing disagreement to the results of Bianchi et al. (14) and Lin et al. (15), both of which did not find lactate in normal appearing brain examinations. The lack of this expected metabolite detection may reflect not only an insufficient brain concentration related to the stage of the disease, but also the failure to probe the proper brain region. In fact, both studies (14,15) evaluated the brain only, avoiding the cerebral spinal fluid (CSF). Potentially, this can be a pitfall because the clearance of lactate into the CSF spaces is slower than the clearance of lactate produced by brain tissue (18). Therefore, there may be a longer window for detection of elevated lactate in CSF which was evident in our study.

Similar sensitivity was also noticed in cases with non specific findings (group II) which have a wide differential diagnosis but by virtue of MRS, mitochondrial disease was suspected and highly considered by scoring system in which all 11 patients included in this group were upgraded from possible into probable grade.

Although there is difference in percentage of detection, yet this is in agreement with the study of Sofou et al. (6) who studied 66 patients with suspected mitochondrial diseases and found lactate peaks in 15 out of the 66 patients with non specific MRI findings.

Although known mitochondrial syndromes have characteristic MRI features, yet MRS still have a role in increasing the specificity of diagnosis by excluding mimics of mitochondrial

diseases as bilirubin toxicity, Wilson disease and disorders of fat metabolism which may mimic Leigh syndrome in showing bilateral basal ganglia T2 hyperintensity, yet show no lactate peaks specially in CSF (10).

All 15 patients included in group I (highly suspected for being syndromic) showed positive lactate on MRS with 6 cases upgraded into higher score mounting to 40% of the studied group (2 from probable into definite and 4 from possible into probable). Our results are running parallel to the older studies of Barkovich et al. (5), Castillo et al. (19), and Wilichowski et al. (20), who found positive lactate in 100% of their studied syndromic mitochondrial diseased patients (14 out of 14 patients). Some difference in percentage of detection was noticed when compared to more recent study of Lin et al. (15), who found positive lactate in only 9 out of 29 patients.

The difference in frequency of detection could be attributed to the fact that the detection of lactate peak on MRS is dependent on the timing or severity of the disease, the location of the affected tissues and the site of interrogation, lactate level of CSF, echo times of MRS, and differences in types of MDs.

It is important to stress that detection of lactate by MRS is not specific for mitochondrial diseases. Increases in CNS lactate are found in several other pathologic processes, such as hypoxia, ischemia, neoplasm, and abscess or inflammation. In most cases, however, clinical presentation and MR imaging appearance would allow unambiguous distinction among the potential diagnoses (15). To overcome the previous limitation which was combined by the absence of final diagnosis due to lack of genetic analysis and muscle biopsy, the role of MRS in the diagnosis of suspected mitochondrial disease was further fortified by applying the forementioned scoring system where 20 patients out of 30 were upgraded after MRS, 18 of which transferred from possible into probable grade and 2 from probable into definite grade. Thus although non specific, and only sharing with one point in the scoring system, when spectroscopic abnormalities appear (in the form of elevated lactate peak) together with a clinical and laboratory framework properly suggestive of mitochondrial dysfunction, they could provide useful additional information regarding such a complex diagnostic puzzle. As a result, they could contribute to reinforcing a diagnostic suspicion and justify further biochemical or genetic investigation.

5. Conclusion

Clinical diagnosis in children with mitochondriopathies is not easy because of variable clinical symptoms. MRS examination is a noninvasive complementary tool for helping to corroborate the diagnosis of MDs, especially in patients exhibiting signal changes on the brain MRI and lactate peaks on MRS but a normal blood lactate level and in those with normal appearing MRI examinations or presenting with non specific MRI findings. Furthermore, it helps determining whether further invasive examinations and/or high-cost diagnostic tests such as muscle biopsy and/or genetic analysis, are needed.

Conflict of interest

None declared.

References

(1) Kim J, Lee SK, Kim EY, Kim DI, Lee YM, Lee JS, et al. Neuroradiologic findings in children with mitochondrial disorder: correlation with mitochondrial respiratory chain defects. Eur Radiol 2008;18(8):1741–8.

(2) Moslemi AR, Darin N. Molecular genetic and clinical aspects of mitochondrial disorders in childhood. Mitochondrion 2007;7(4):241–52.

(3) Finsterer J. Central nervous system manifestations of mitochondrial disorders. Acta Neurol Scand 2006;114:217–38.

(4) Chi CS, Lee HF, Tsai CR, Chen WS, Tung JN, Hung HC. Lactate peak on brain MRS in children with syndromic mitochondrial diseases. J Chin Med Assoc 2011;74(7):305–9.

(5) Barkovich AJ, Good WV, Koch TK, Berg BO. Mitochondrial disorders: analysis of their clinical and imaging characteristics. AJNR 1993;14(5):1119–37.

(6) Sofou K, Steneryd K, Wiklund LM, Tulinius M, Darin N. MRI of the brain in childhood-onset mitochondrial disorders with central nervous system involvement. Mitochondrion 2013;13(4):364–71.

(7) Barragan-Campos HM, Vallee JN, Lo D, Barrerea-Ramirez CF, Argote-Greene M, Sanchez-Guerrero J, et al. Brain magnetic resonance imaging findings in patients with mitochondrial cytopathies. Arch Neurol 2005;62(5):737–42.

(8) Bianchi MC, Sgandurra G, Tosetti M, Battini R, Cioni G. Brain magnetic resonance in the diagnostic evaluation of mitochondrial encephalopathies. Biosci Rep 2007;27:69–85.

(9) Saneto R, Friedman S, Shaw D. Neuroimaging of mitochondrial disease. Mitochondrion 2008;8:396–413.

(10) Haas R, Dietrich R. Neuroimaging of mitochondrial disorders. Mitochondrion 2004;4:471–90.

(11) Morava E, Van Den Heuvel L, Hol F, De Vries MC, Hogeveen M, Rodenburg RJ. Mitochondrial disease criteria: diagnostic applications in children. Neurology 2006;67(10):1823–6.

(12) Barkovich AJ. An approach to MRI of metabolic disorders in children. J Neuroradiol 2007;34(2):75–88.

(13) Chi CS, Lee HF, Tsai CR, Chen CC, Tung JN. Cranial magnetic resonance imaging findings in children with nonsyndromic mitochondrial diseases. Pediatr Neurol 2011;44(3):171–6.

(14) Bianchi MC, Tosetti M, Battini R, Manca ML, Mancuso M, Cioni G, et al. Proton MR spectroscopy of mitochondrial diseases: analysis of brain metabolic abnormalities and their possible diagnostic relevance. AJNR 2003;24(10):1958–66.

(15) Lin DD, Crawford TO, Barker PB. Proton MR spectroscopy in the diagnostic evaluation of suspected mitochondrial disease. AJNR 2003;24(1):33–41.

(16) Dinopoulos A, Cecil KM, Schapiro MB, Papadimitriou A, Hadjigeorgiou GM, Wong B, et al. Brain MRI and proton MRS findings in infants and children with respiratory chain defects. Neuropediatrics 2005;36(5):290–301.

(17) Nissenkorn A, Zeharia A, Lev D, Fatal-Valevski A, Barash V, Gutman A, et al. Multiple presentations of mitochondrial disorders. Arch Dis Child 1999;81:209–15.

(18) Inao S, Marmarou A, Clarke GD, Andersen BJ, Fatouros PP, Young HF. Production and clearance of lactate from brain tissue, cerebrospinal fluid, and serum following experimental brain injury. J Neurosurg 1988;69:736–44.

(19) Castillo M, Kwock L, Green C. MELAS syndrome: imaging and proton MR spectroscopic findings. AJNR 1995;16:233–9.

(20) Wilichowski E, Pouwels PJ, Frahm J, Hanefeld F. Quantitative proton magnetic resonance spectroscopy of cerebral metabolic disturbances in patients with MELAS. Neuropediatrics 1999;30:256–63.