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Mitochondrial leukoencephalopathies: A border zone between acquired and inherited white matter disorders in children?

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ABSTRACT

Background: There is emerging evidence implicating mitochondrial dysfunction in the pathogenesis of acquired demyelinating disorders such as multiple sclerosis. On the other hand, some of the primary mitochondrial disorders such as mitochondrial leukoencephalopathies exhibit evidence of neuroinflammation on MRI. The inter-relationship between mitochondrial disorders and episodic CNS inflammation needs exploration because of the therapeutic implications.

Objective: We sought to analyze the clinical course and MRI characteristics in a cohort of patients with mitochondrial leukoencephalopathy to determine features, if any, that mimic primary demyelinating disorders. Therapeutic implications of these findings are discussed.

Patients and methods: Detailed analysis of the clinical course, magnetic resonance imaging findings and therapeutic response was performed in 14 patients with mitochondrial leukoencephalopathy. The diagnosis was ascertained by clinical features, histopathology, respiratory chain enzyme assays and exome sequencing.

Results: Fourteen patients [Age at evaluation: 2–7 yrs, M: F-1:1] were included in the study. The genetic findings included variations in *NDUFA1* (1); *NDUFV1* (4); *NDUFS2* (2); *LYRM* (2);*MPV17*(1); *BOLA3*(2); *IBA57*(2). Clinical Features which mimicked acquired demyelinating disorder included acute onset focal deficits associated with encephalopathy [10/14, 71%], febrile illness preceding the onset [7/14, 50%] unequivocal partial or complete steroid responsiveness [11/11], episodic/ relapsing remitting neurological dysfunction [10/14, 71%] and a subsequent stable rather than a progressive course [12/14, 85%]. MRI characteristics included confluent white matter lesions [14/14, 100%], diffusion restriction [11/14,78.5%], contrast enhancement [13/13,100%], spinal cord involvement [8/13,61.5%], lactate peak on MRS [13/13] and white matter cysts [13/14, 92.8%].

Conclusion: Clinical presentations of mitochondrial leukoencephalopathy often mimic an acquired demyelinating disorder. The therapeutic implications of these observations require further exploration.

1. Introduction

White matter involvement is increasingly being recognized as a manifestation of mitochondrial disorders and the term mitochondrial leukoencephalopathy or leukodystrophy has been used to designate these disorders (Kevelam et al., 2016). They are mainly defined by the MRI characteristics such as cystic lesions in the abnormal white matter, additional gray matter lesions, restricted diffusion, contrast enhancement, and elevated lactate on magnetic resonance spectroscopy of the brain (van der Knaap MS, 1995). Clinically, patients with mitochondrial leukoencephalopathy most often present with monophasic or recurrent episodes of neurological regression (Dallabona et al., 2016). Acute onset neurological deficits in combination with large confluent white matter lesions on MRI often lead to diagnosis of an acquired

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Table 1
Clinical and MRI Features during the episodes in children with mitochondrial leukoencephalopathy.

Pt No/Gender	Episodes	Age at presentation	Clinical features	MRI characteristics [T2/FLAIR signal changes/DWI/CE/MRS]	Immunomodulation &other treatment	Response
Patient 1/M	1st episode	2.5yrs	Febrile illness, vomiting, encephalopathy, ataxia	Multifocal discrete hyperintense lesions in bilateral supratentorial white matter, thalami and left cerebellar hemisphere with nodular CE	Inj. MP X 5days, oral steroids taper X 4weeks	Improved, no residual deficits
	2nd episode	4.5yrs	Fever,vomiting, seizures, encephalopathy, left hemiparesis, ataxia	Bilateral temporo- parietal, occipital and frontal regions in deep white matter and subcortical zones, patchy CE, no restricted diffusion, moderately increased lipid lactate peak	Inj.MP X 5days, oral steroid taper	Improved, no residual deficits
	Follow up	4yrs& 10mo	Asymptomatic period	Residual bright signals in bilateral peritrogonal white matter and scattered focal hyperintense lesions in bilateral corona radiate and subcortical regions of fronto-parietal lobes, no CE	Nil	No deficits
	3rd episode	5yrs	Febrile illness, Rt hemiparesis, Rt focal seizure, aphasia	New areas of bright signals in deep white matter in left parietal lobe & subcortical region in left occipital lobe resembling tumefactive demyelination. CE +	Inj.MP X 5days, oral steroid taper for three weeks	Improved, no deficits
	4th episode	5yrs 5mo	Sub acute onset, Lt focal seizures,visual agnosia,irritability	Bilateral fronto parieto temporal and occipital lesions on left > Rt. New areas of T2 bright signals involving left half of midbrain &pons. CE+	Inj.MP X 5days followed by steroid taper for three weeks	Improved
	Follow up MRI after one month in the asymptomatic period			Interval MRI-Persisting signal changes in the bilateral fronto-parieto-temporal and occipital region and left half of pons. Reduction in the extent and mass effect Two new enhancing nodular lesions seen in left frontal region	Started on Inj.Interferon x 4 months	Asymptomatic
	5th episode	5yrs 8mo	Left sided focal seizures	Conflent hyperintensities in periventricular white matter in occipital, frontal and temporal regions, Lactate peak, no CE, no restricted diffusion	Inj. MPX 5days monthly X 6mo once in two months X 6months, once in three months X 1 dose	No episodes for 1.5 yrs
	Follow up MRI in the asymptomatic period after six months			Confluent asymmetrical lesions in bifrontal and biparietal regions, no diffusion restriction, no CE, Lactate peak+	Nil, On multivitamins	No further episodes
	6th episode	6.5yrs	Rt focal seizures, Rt hemiparesis	Confluent white matter lesions in frontal, parietal and temporal lobes, No CE or restriction,SC +	Inj.MPX5days	Improved
	Follow up	10yrs	No neurological deficits	ND	High dose vitamins	Stable course
Patient 2/ F	1st episode	3yrs	Febrile illness, loss of mile stones, encephalopathy	Confluent white matter lesions in frontal parietal and temporal lobes with rarefaction, CE + Corpus callosal lesions- splenium and body	Inj.MPX 5days	Full improvement
	2nd episode	One month after	Encephalopathy	ND	Inj.MP monthly pulse dosesX6 months	Partial improvement
	Follow up	6 years	GDD, optic atrophy, seizures, spastic parapresis	Bilateral symmetrical nonhomogeneous signal changes in cerebral white matter, rarefied appearance +, cysts+, SC+, restricted diffusion, lactate peak	Nil. Maintained on high dose vitamins	Progressive course
Patient3/M	1st episode	9 mo	Encephalopathy, regression	Multifocal confluent white matter signals in frontal parietal and temporal region, rarefied appearance of white matter, restricted diffusion along the edges	Inj.MP X 5days, steroids taper X 6 weeks	Partial response, toe walking
	2nd episode	2yrs	Febrile illness, loss of mile stones, seizures, pyramidal signs	Confluent hyperintense lesions bilateral periventricular and subcortical white matter, putamen, substantia nigra, medial thalamus, SC, white matter cysts, lactate peak, restricted diffusion, CE+, SC signal changes Corpus callosum is affected in full extent.	Inj.MP X5 days.	Improvement
	Follow up	3yrs	Ambulent, spastic paraparesis, optic atrophy	ND	Nil. On high dose vitamins	No further episodes
Patient 4, F	Insidious onset	6mo	Diarrhoeal illness, loss of mile stones, seizures, family history positive	large confluent bilateral hyperintense signal changes in white matter lesions restricted diffusion, CE+, spinal cord signal changes,white matter cysts-present, Lactate peak	Not received, high dose vitamins	Spastic paraparesis, Optic atrophy
	Follow up	3yrs	No further episodes, gaining mile stones	ND	High dose vitamins	
Patient 5, M	Insidious onset	9mo	Loss of acquired mile stones	Large multifocal confluent hyperintense lesions, restricted diffusion& CE, SC signal changes	Inj.MPX 5 days followed by oral steroids X one year	Fully improved
	Follow up	10yrs	Achieved independent walking, running, language delay	Confluent lesions in bifrontal, parietal and occipital lobes Lt temporal lobe, restricted diffusion and CE, multiple cysts	Nil, on high dose vitamins	Poor scholastic performance

(*continued on next page*)

Table 1 (*continued*)

Pt No/Gender	Episodes	Age at presentation	Clinical features	MRI characteristics [T2/FLAIR signal changes/DWI/CE/MRS]	Immunomodulation &other treatment	Response
Patient 6,M	Ist episode	9mo	Fever, seizures, regression of mile stones	Multiple large confluent T2/FLAIR lesions, restricted diffusion, CE, SC signal changes	Inj.MP X 5days	Complete improvement
	2nd episode	13mo	Fever seizures, regression, pyramidal signs, optic atrophy	ND	Inj.MPX5 days	Partial response, spastic paraparesis
Patient 7,F	Insidious onset	6mo	One episode of neuroregression, seizures, pyramidal signs, optic atrophy	Multiple large confluent symmetrical signal changes in white matter, restricted diffusion, CE, white matter cysts, lactate peak	Inj.MPX5 days	Partial response, spastic paraparesis
Patient 8, F	1st episode	8mo	Febrile illness, Visual loss, irritability, neuroregression	Multifocal discrete and confluent lesions, restricted diffusion, CE, SC lesions, lactate peak	Inj.IVIG X 5days, oral steroids.	Complete improvement
	2nd episode	2yrs	Seizures, visual loss, irritability, neuroregression	Multiple focal and confluent lesions, restricted diffusion,	Inj.MP X 5days.	Partial improvement
Patient 9, M	Ist episode	5.5 yrs	Lt hemiparesis progressing to quadriparesis in few days. No encephalopathy	Multifocal confluent lesions white matter, restricted diffusion, patchy CE, multiple cysts, Lactate peak present	Inj.MP X 5 days	complete improvement
	2nd episode	6yrs	Hemiparesis progressing to quadriparesis. No encephalopathy	ND	InjMPX5days	Improved
	3rd episode	7yrs	Lt hemiparesis	ND	Inj.MPX5days	Improved
	4th episode	7.5 yrs	Suddden bilateral vision loss	Symmetrical confluwnt cystic white matter lesions in periventricular region, cervical spional cord lesions	Inj.MPX5days	Improved
	5th episode	8yrs	Febrile illness, quadriparesis	NA	NA	Expired
Patient 10, F	1st episode	2.5 yrs	Febrile illness, Jaundice, Gait difficulty, falls followed by ataxia, quadriparesis, recurrent unexplained vomiting	Multifocal confluent lesions in fronto parietal region with central hypointensity on FLAIR, restricted diffuson & enhancement. Spinal cord signal changes present	Inj. MPX 5 days followed by oral steroids for 6 months	Distinct steroid responsiveness
	2nd episode	3.5yrs	Insidious onset lower limb weakness	ND	Oral steroidsX1month	Improved
	3rd episode	6yrs	Bulbar symptoms/ progressive slurring of speech	Multifocal confluent white matter lesions in fronto parietal region. Restricted diffusion present, CE+	Inj.MPX5days,oral steroids for one month	Minimal improvement, spasticity of LL
	Follow up	11 yrs	Spastic quadriparesis, seizures, bedridden status	Symmetrical confluent white matter signal changes with cysts and bilateral dentate nuclei and cerebellar white matter hyperintensities, SC signal changes +	Not received.On High dose vitamins	spastic quadriparesis
*Patient 11, F	1st episode	18mo	Insidious onset regression, gait difficulty falls Improved over next	Signal changes in periventricular and deep white matter, diffusion restriction, contrast enhancement	Not Received	Spastic paraparesis
	2nd episode	2.5yrs	Febrile illness, transient regression	ND	Inj.MP X 5days	Partial improvement
*Patient 12, F	Onset	1.5yrs	Minor head injury followed by insidious onset gait difficulty, recurrent falls,speech regression, pyramidal signs	Fronto-parietal lesions	Not received	Mild spastic paraparesis, independent in all daily activities
	Follow up	12yrs	Spastic paraparesis	Posterior periventricular and deep white matter signal changes, Lactate peak	Nil, on high dose vitamins	Stable course, attends school, IDD
Patient 13, M	1st episode	18 mo	Febrile illness, loss of acquired mile stones, difficulty in standing and walking	Large confluent hyperintense lesions in frontal parietal and occipital regions with restricted diffusion& CE, Lactate peak	Inj.MP X 5days	Partial improvement
	2nd episode	19mo	Bulbar weakness, quadriparesis	ND	Inj.MPX5 days followed by oral steroids for 2 weeks	Gradual improvement, spastic paraparesis
Patient14, M	1st episode	20 mo	Febrile illness, Rt hemiparesis progressing to quadriparesis	Large confluent hyperintense lesions in frontoparietal white matter; genu, splenium and anterior part of the body of corpus callosum, multiple white matter cysts, restricted diffusion, lactate peak	Inj.MP X 5 days followed by oral steroids	Partial improvement
	2nd episode	22mo	Progressive loss of walking and language mile stones	ND	Inj X MP pulse doses for three months	Improvement in motor cognitive and social mile stones
	Follow up	31mo	Spastic paraparesis	Bilateral symmetrical hyperintensities in periventricular corona radiata and centrum semi ovale, cysts increased in number. No diffusion restriction or CE	High dose vitamins	No further episodes

Abbreviations: CE- contrast enhancement; DWI-Diffusion weighted; ; GDD- Global developmental delay; IDD- Intellectual disability; Inj.MP- Methyl Prednisolone injections; mo -months; Lt- left; MRS-Magnetic resonance spectroscopy; NA- Not available; ND-Not done; Rt- Right; SC-Spinal cord

* siblings

demyelinating disorder such as acute disseminated encephalomyelitis (ADEM). In some disorders, predominant visual impairment and white matter lesions may suggest diagnosis of neuromyelitis optica spectrum disorders, which are further emphasized by the presence of spinal cord signal changes on MRI.

On the other hand, it is increasingly being evident that mitochondrial abnormalities are involved in the development and progression of multiple sclerosis (MS) (Mao and Reddy, 2010). The most compelling evidence implicating the role of mtDNA comes from the observation of susceptibility of LHON [Leber's hereditary optic neuropathy] patients to develop white matter lesions resembling MS (Matthews et al., 2015). However studies interrogating the presence of LHON-associated mutations in patients with multiple sclerosis (Hanefeld et al., 1994; Kalman et al., 1995) as well as those probing an increased MS risk in particular mitochondrial haplogroups (Ban et al., 2008; Kalman et al., 1999; Otaegui et al., 2004; Tranah GJ et al., 2015), have revealed conflicting results. An exploratory study on the mitochondrial DNA variations and haplogroups in children with acquired demyelinating syndromes (ADS) have raised the possibility that mtDNA variants or haplogroups may influence the age at onset and subsequent MS risk (Venkateswaran et al., 2011). These observations suggest that the link between mitochondrial dysfunction and ADS is unclear and needs to be explored further.

Importance of MRI in the interpretation and diagnosis of mitochondrial leukoencephalopathies has been already emphasized. Even though the characteristics of mitochondrial leukoencephalopathy have been highlighted in literature, the therapeutic implications of these findings still needs to be elucidated. This study analyzed the clinical and MRI characteristics in a cohort of children with mitochondrial leukoencephalopathy so as to define the features that mimic acquired demyelinating disorders.

1.1. Patients and methods

The cohort was derived from a database of patients who underwent exome sequencing as part of a study on phenotype genotype correlations in mitochondrial disorders, over a period of two years (2015–2017). The institute ethics committee approved the study and all subjects gave written informed consent.

1.2. Phenotypic characterization

Patients were recruited into the study if they satisfied the clinical criteria of mitochondrial disorder as defined by Bernier et al. (Bernier et al., 2002) and a comprehensive evaluation, including estimation of serum lactate, muscle histopathology, assay of respiratory chain complex enzymes, brain magnetic resonance imaging, nerve conduction studies, electroencephalography (EEG) and evoked potential studies suggested a probable diagnosis of mitochondrial disorder. Exome sequencing was performed using illumina sequencing platform and the gene panel consisted of 6440 genes inclusive of all nuclear-encoded mitochondrial genes that are strongly associated with a disease on OMIM (Online Mendelian Inheritance in Man). The details are provided in the supplementary file.

Among the 85 patients who underwent exome sequencing, 36 showed variations in mitochondrial disease related genes. Among these, 14 patients (Age range: 2–7yrs, M: F- 1:1) displayed significant white matter involvement and qualified for a diagnosis of mitochondrial leukoencephalopathy and were included in final analysis. Their clinical features, MRI findings and therapeutic responses were analyzed retrospectively. All patients had significant white matter hyperintensities involving one or more of the white matter zones viz. periventricular, deep white matter and subcortical white matter as well as involvement of multiple lobes [frontal, parietal, temporal and occipital white matter]. The sequences analyzed included T1 weighted (T1W), T2 weighted (T2W) and Fluid attenuated inversion recovery (FLAIR) sequences in all. Additional sequences included diffusion weighted images (DW1, n = 13), Contrast images (n = 13) & Magnetic resonance spectroscopy (n = 13). Majority of the children received evaluation and treatment during the acute phase in peripheral hospitals. The information on the CSF studies and the details of the immunomodulation were retrieved from the referral notes and treating physician's notes. All patients were evaluated and followed up by the same clinical team [PSB, ABT, MN &SS]. MRI findings were independently reviewed by two neurologists (PSB &ABT) and one neuroradiologist (HRA). Descriptive statistics were used to describe the key findings. The comparison of proportions in different groups were done by t- test.

1.3. Results

The detailed clinical features, MRI characteristics, and immunomodulation and follow up are provided in Table 1. The genetic findings included variations in *NDUFA1*(1); *NDUFV1*(4); *NDUFS2* (2) ; *LYRM* (2); *MPV17*(1); *BOLA3*(2); *IBA57*(2). The details of the genetic findings are provided in the Supplementary table.

1.4. Clinical features

The age at onset of the symptoms in the patients ranged from 6 months to 5.5 years (Mean ± SD − 1.67 ± 1.3 yrs). In six children age of onset was in infancy. The period of follow up ranged from 1 to 7 years and the mean duration of illness at last follow up was 4.5 ± 3.6 years. Majority had an acute presentation (n = 10). History of an inciting event was present in 10 [febrile illness, n = 8; jaundice, n = 1; minor head trauma, n = 1]. Infants in the cohort manifested with regression of acquired milestones. Neurological examination showed pyramidal signs in all and additional ataxia in four. Primary optic atrophy was present in eight (57%). Even though visual loss was the presenting manifestation in two patients optic disc swelling was not reported. Except for three patients who had insidious onset of symptoms all received immunomodulation presuming a diagnosis of an acquired demyelinating syndrome and most common diagnosis considered was acute disseminated encephalomyelitis. The response to immunomodulation was either partial or complete. In those with partial response the residual deficits were spasticity in the lower limbs and mild incoordination of the upper limbs. Even those patients, who did not have overt spasticity, had pyramidal signs in lower limbs.

1.5. Relapses

Details of relapses are provided in Table 1. Ten patients had multiple episodes (median number of episodes −2). For seven patients the second episode occurred within 2 months of the first episode. Three children had multiple episodes [patient 1,9 &10]. While the episodes were heralded by seizures in patient 1, there were no seizures in patient 9 and 10.

1.6. Cerebrospinal fluid (CSF) examination

Results of the cerebrospinal fluid examination during the acute presentation was available in 11 patients. Pleocytosis was noted in none while elevated CSF protein was noted in three.

1.7. Magnetic resonance imaging findings

Description of the MRI findings is provided in Table 1 and summary is provided in Table 2. MRI findings included large confluent white matter signal changes that showed diffusion restriction and patchy contrast enhancement in the acute phase and on follow up, in some patients. In those children with discrete lesions in the initial scans, the signal changes tended to become confluent and bilaterally symmetrical on follow up images (Fig. 1A-C). Cysts inside the white matter were

Table 2
Comparison of clinical and mri features in patients with mitochondrial leukoencephalopathy with cohorts of patients with ADEM.

Parameters	Mito LE (Present study)	ADEM-India (Same institute)	ADEM- India (Singhi et al.)	ADEM-Japan (Yamaguchi et al.)	ADEM-UK (Absoud et al.)
No of patients	N = 14	N = 35	N = 52	N = 66	N = 40
Mean Age at onset	1.67 ± 1.3 yrs	8.2 ± 4.1 yrs*	6.14 ± 3.17yrs*	5.5 ± 3.8 yrs*	Median age − 5.3yrs
Gender [M: F]	1:1	1.2:1	2.7:1	2:1	1.5:1
Febrile illness	7 [50%]	31 [88.8%]*	13 [25%]	45/66[68%]	NA
Neurological Features					
Encephalopathy	9 [64.2%]	35 [100%]	Majority	66/66[100%]	40[100%]
Pyramidal signs	14 [100%]	13 [37%]*	42 [80.7%]	NA	24 [60%]*
Ataxia	7 [50%]	8 [25%]	6 [11.5%]*	NA	18 [45%]
Visual loss	3 [21.4%]	5 [14%]	11 [21.2%]	7/66[11%]	3 [0.1%]*
Optic atrophy	8 [57%]	NA	NA	NA	NA
Seizures	8 [57.1%]	8 [22%]*	19 [36.5%]	21 [32%]	8 [20%]*
CSF pleocytosis	Nil	18 [32%]	Majority	56/66[85%]	23[58%]
MRI Findings					
Periventricular lesions	14 [100%]	11 [31%]*	NA	20/66[30%]*	9 [22.5%]*
Lobar/DeepWM	14 [100%]	18 [51%]*	NA	NA	24 [60%]*
Subcortical/ Juxtacortical	6 [15%]	29 [68%]*	NA	41/61[67%]*	20 [50%]*
Cerebral cortex	Nil	7 [20%]	NA	28/61 [46%]	14 [35%]
Corpus callosum	14 [100%]	9 [25.7%]*	7 [13.5%]*	11/61 [18%]*	3 [0.1%]*
Thalamus	2 [14.3%]	8 [23%]	16 [30.8%]	30/61[49%]*	24 [60%]*
Basal Ganglia	1 [7.0%]	6 [17%]	9 [17.3%]		
Cerebellum	4 [28.6%]	9 [25.7%]	14 [26.9%]	20/66[30%]	17 [42%]
Brainstem	2 [14.3%]	14 [40%]	9 [17.3%]	19/66[29%]	22 [55%]*
Spinal cord	8 [61.5%]	4/6 [66%]	5 [9.6%]*	16/42[38%]	8/12[67%]
Contrast enhancement	13/13[100%]	13/27[48%]*	5 [48%]*	NA	NA
Diffusion restriction	11/14[78.5%]	8/26 [30%]*	NA	NA	NA
Lactate peak on MRS	13/13 [100%]	NA	NA	NA	NA
White matter cysts	13/14 [92.8%]	NA	NA	NA	NA
Residual deficits	12 [92.3%]	2[5.7%]*	20[38.7%]*	11 [16.7%]*	NA
Death	1/13[7.6%]	1/35[2.9%]	none	none	1 [2.5%]

* P < 0.05, Abbreviations: ADEM-Acute disseminated encephalomyelitis; CSF- cerebro spinal fluid; MRS- Magnetic resonance spectroscopy; NA-Not available;WM-white matter.

evident in all except one, either at the time of acute presentation or on follow up (Fig. 2B). Corpus callosal involvement was prominent in all but involvement of internal capsule, brainstem and pyramidal tract were seen in only one patient each. Likewise the presence of signal changes in basal ganglia, thalamus and dentate was seen in only one patient each.

1.8. Clinical and MRI findings in mitochondrial LE vs. acquired demyelinating syndrome

The clinical features and MRI findings in this cohort was compared with that of children with acute disseminated encephalomyelitis (ADEM). The cohorts included 35 children with ADEM presented to our institute as well as with another cohort from India (Singhi et al., 2006) and two recent series on ADEM from Japan (Yamaguchi et al., 2016), and UK (Absoud et al., 2013). The age at onset, clinical features and MRI findings were compared and provided in Table 2. The patients with mitochondrial LE presented at a significantly younger age and had more chance of having residual motor deficits compared to children with ADEM (p value < 0.05). Comparison of MRI features showed that presence of periventricular and deep white matter lesions, corpus callosal lesions, contrast enhancement and restricted diffusion were significantly higher compared to children with ADEM (p value < 0.05). In contrast presence of subcortical/juxta cortical lesions were less common compared to ADEM. Even though the details of MRS and white matter cysts were not available for comparison in the ADEM series, both the features were consistently seen in mitochondrial LE.

1.9. Clinical and MRI findings in mitochondrial LE vs other mitochondrial disorders

Clinical phenotypes in the rest of the children with mitochondrial disorders included Leigh and Leigh like syndrome (n = 15), encephalomyopathy (n = 2), chronic progressive external ophthalmoplegia with epilepsy (n = 4), and mitochondrial neurogastrointestinal encephalopathy (MNGIE, n = 1). The MRI findings included bilateral

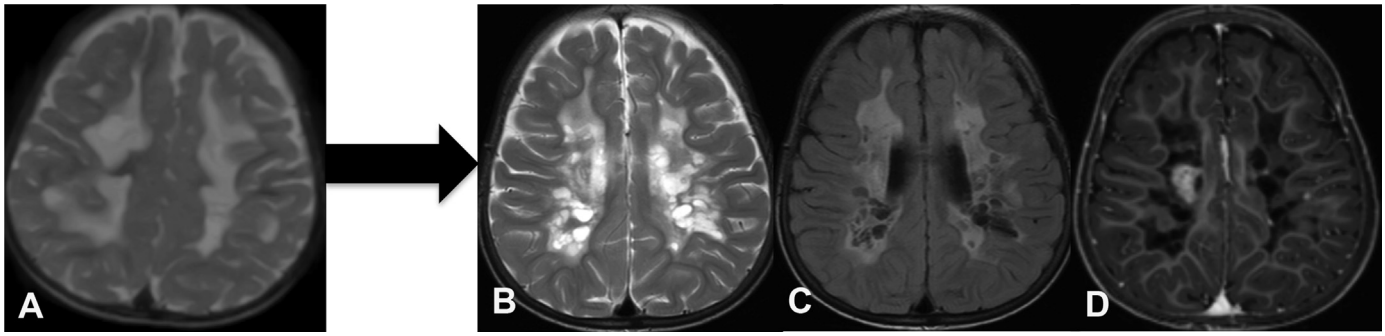

Fig. 1. MRI Brain in a 2-year-old child with Mitochondrial leukoencephalopathy and mutations in IBA57 (Patient 14). A) T2W axial image at the time of first presentation shows large asymmetrical confluent white matter signal changes B-D) Follow up study shows rarefied white matter on T2W axial view (B), cysts inside the affected white matter on FLAIR(C), contrast enhancement (D).

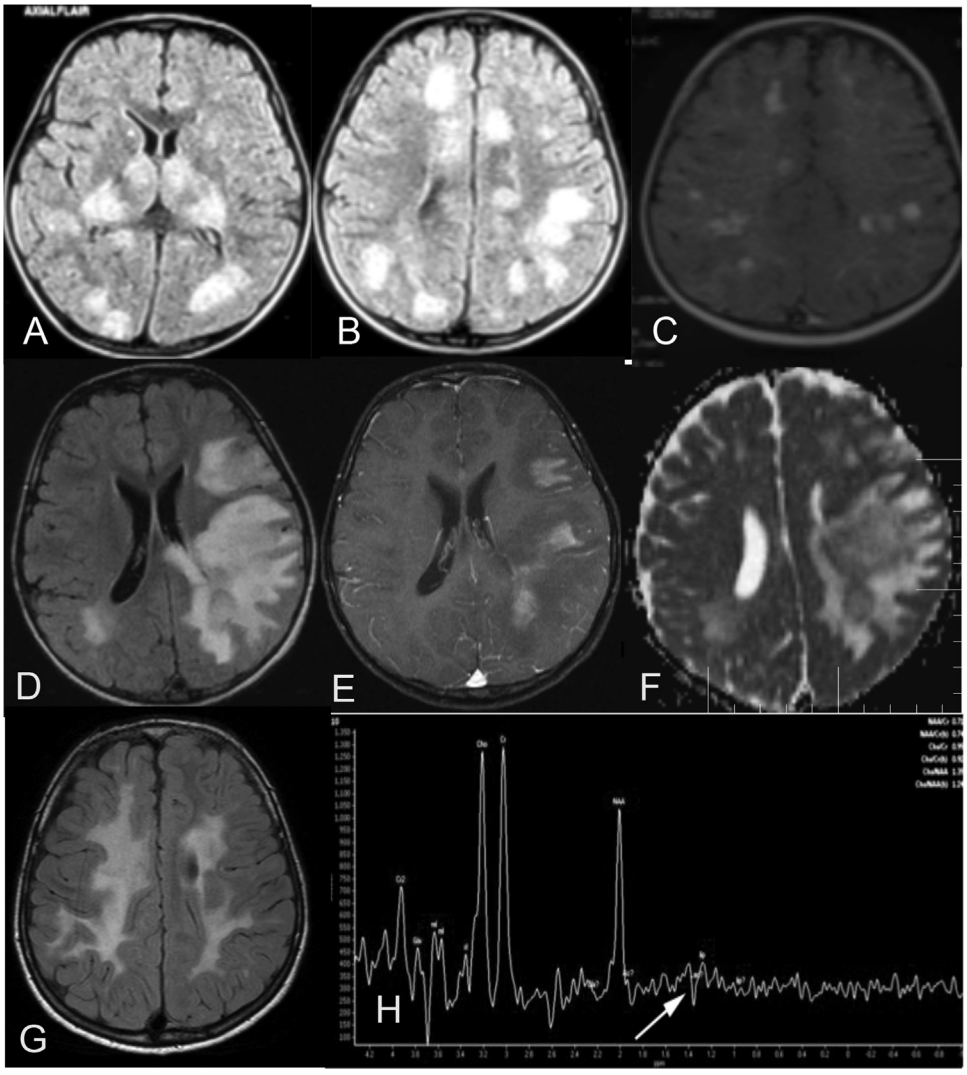

Fig. 2. MRI brain in patient 1 with NDUFA1 variation. A-C –MRI at 2.5yrs at the time of initial presentation shows multiple discrete white matter and gray matter T2/FLAIR hyper intensities with contrast enhancement. D-F- MRI during the third episode at the age of 4.5 yrs shows large asymmetrical white matter lesions with contrast enhancement and patchy restricted diffusion. G. The lesions shows tendency to become symmetrical and diffuse on follow up imaging at 7.5yrs H. MRS shows lactate peak.

symmetrical signal changes in the basal ganglia, brain stem and cerebellum (n = 15) predominantly noted in children with Leigh and Leigh like syndrome. Normal MRI findings were noted in children with encephalomyopathy and chronic progressive external ophthalmoplegia. The child with MNGIE had bilateral symmetrical T2/FLAIR signal changes involving the periventricular and deep white matter without any contrast enhancement or diffusion restriction. Subcortical fibers were spared. There was presence of lactate peak on MRS.

The detailed case history and investigation results including brain biopsy findings of one of the patients is described below to demonstrate the diagnostic and therapeutic challenge posed by these patients.

Patient 1: This 10 year old boy of Indian origin was the first child of non-consanguineous parents with normal birth history and developmental milestones. He was apparently normal till 2.5 years when he developed ataxia, seizures and encephalopathy following a febrile illness. CSF study was normal. MRI demonstrated T2/FLAIR hyper intense lesions involving both gray and white matter with contrast enhancement [Fig. 2A-C]. He received pulse methyl prednisolone for presumptive diagnosis of acute disseminated encephalomyelitis and made complete clinical recovery. Thereafter he presented with multiple relapsing remitting neurological episodes characterized by seizures, hemiparesis and ataxia associated with relapsing remitting white matter lesions on MRI [Table 1, Fig. 2D-H]. He was referred to our institute at the age of 6 years during the fifth episode.

Review of the evaluations done elsewhere revealed high serum alanine, lactic acid metabolites on urinary organic acid estimation and an elevated serum lactate on multiple occasions. Muscle biopsy showed complex I deficiency on respiratory chain enzyme assays. Complete mitochondrial DNA sequencing revealed only polymorphisms. Targeted sequencing of complex 1 nuclear genes revealed a previously reported hemizygous variation in *NDUFA1* (c.94G > C, p. G32R), which was confirmed by Sanger sequencing. His mother and sister carried the same variation.

Biopsy from the right frontal cortex revealed a small fragment of cortex with white matter (Fig. 3A). Extensive loss of myelin was seen on Luxol Fast Blue stain (Fig. 3B). A few preserved strands of myelinated axons traversing the white matter was seen. In contrast, there was relative preservation of axonal tracts in the demyelinated segment (Fig. 3C). Tissue response in the form of scattered CD68 labelled ramified microglia and few clusters of histiocytes were detected within the zone of demyelination (Fig. 3D), in addition to fibrillary gliosis and several hypertrophic reactive astrocytes (Fig. 3F). There was no perivenular demyelination or foamy histiocytes.

Prior to presentation to us, he had received methyl prednisolone injection during each episode followed by short and tapered steroid treatment. He also received Inj. Interferon for a brief period of time presuming a diagnosis of pediatric MS. In view of the relapsing remitting neurological episodes responsive to steroid therapy, the initial diagnosis considered was an acquired demyelinating disorder. In view of the multiple relapses, patient was initiated on monthly pulses of methyl

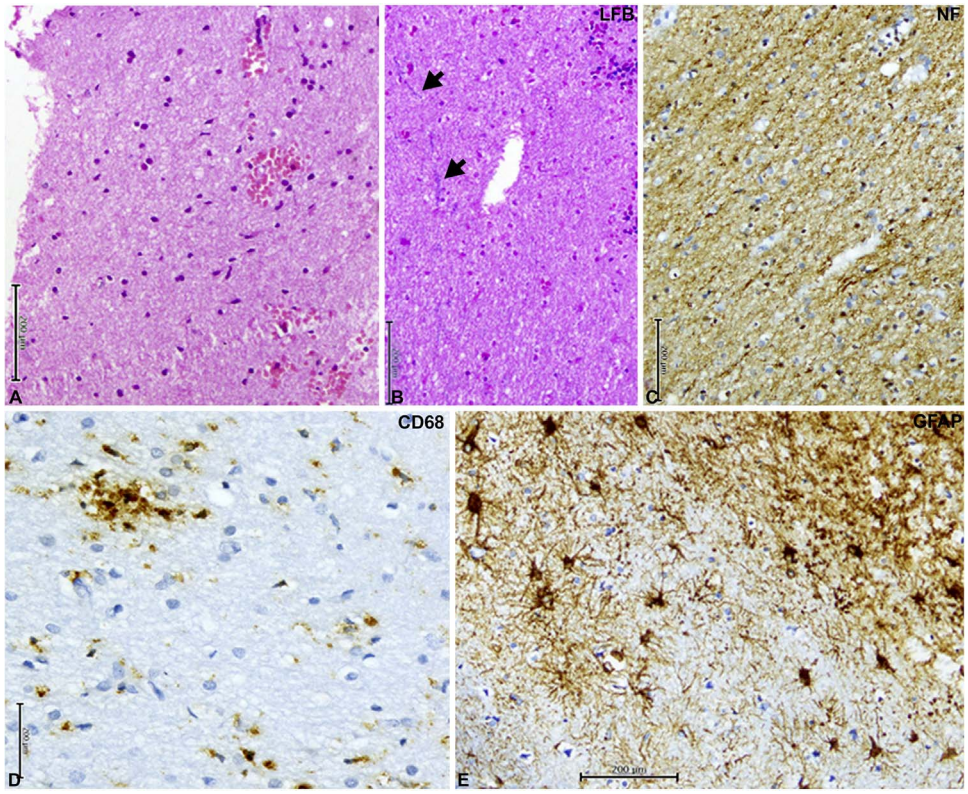

Fig. 3. Brain biopsy findings in Patient 1 (A- F): Brain biopsy included a small fragment of right frontal cortex with white matter (A) which reveals extensive loss of myelin (B). Note few preserved strands of myelinated axons traversing the white matter (arrows, B). In contrast, there is relative preservation of axonal tracts in the demyelinated segment (C). Tissue response in the form of scattered CD68 labelled ramified microglia and few clusters of histiocytes are seen within this zone (D) and several hypertrophic reactive astrocytes with fibrillary gliosis (F). [A:H&E; B: Luxol Fast Blue (LFB); C: Neurofilament; D: CD68; F:GFAP. Magnification = scale bar (200 µm)].

prednisolone. He also received high dose vitamins. After the genetic report, the patient was maintained only on high dose vitamins. The patient remained relapse-free thereafter and is being maintained on high dose vitamins from the age of eight years.

2. Discussion

We have described a cohort of children with mitochondrial leukoencephalopathy with special reference to clinical course, therapeutic response and MRI findings. Even though there are descriptions on clinical and MRI features of mitochondrial leukoencephalopathies in the literature, the specific evidence of neuroinflammation with therapeutic implications are relatively unknown highlighting the novelty of the present study.

The diagnoses most often considered by the referring physicians were acute disseminated encephalomyelitis (ADEM) or multiphasic ADEM. This was substantiated by the presence of acute onset focal deficit associated with encephalopathy as defined by international pediatric multiple sclerosis criteria (Gordon-Lipkin and Banwell, 2017; Krupp et al., 2013). History of febrile illness preceding the onset, unequivocal steroid responsiveness, and a subsequent stable course albeit with deficits rather than a progressive degenerative course also corroborated the diagnosis of an acquired demyelinating disorder. However, comparison of the clinical features with other cohorts of children with ADEM brought out the differences from the primary demyelinating disorder. One of the important differentiating features was an early age of presentation as compared to children with primary demyelinating disorder. The usual age of onset of ADEM in children ranges from 5 to 8 years (Gordon-Lipkin and Banwell, 2017; Krupp et al., 2013). On the other hand patients with mitochondrial LE presented either in the infantile or late infantile period and some of them had insidious rather than acute presentation. The second point pertains to the presence of primary optic atrophy in many patients at the time of initial presentation. Even though the information on optic atrophy was not available for comparison in the demyelination groups, primary optic atrophy was present in more than half of the patients in this series at the time of presentation. Thirdly, children with mitochondrial LE most often had residual motor deficits on follow up even though they showed complete or partial clinical response to steroids in the acute phase. On the other hand residual motor deficits are less commonly reported in ADEM (Absoud et al., 2013; Yamaguchi et al., 2016). Seizures were a major part of the episodes and sometimes the heralding event in some patients as exemplified in the patient with *NDUFA1* variation. The difference was significant compared to two out of three ADEM cohorts and may be another useful differentiating point.

The clinical features in other mitochondrial phenotypes and that of mitochondrial leukoencephalopathy also showed distinct differences. Systemic features such as peripheral neuropathy, auditory involvement, and myopathic features were absent in children with leukoencephalopathy. The clinical features were related to long tract involvement in comparison to other phenotypes. This also may pose a major diagnostic dilemma for suspecting a mitochondrial etiology in patients primarily presenting with neuroregression and leukoencephalopathy. But familiarity with the MRI patterns compared to other leukodystrophies may help the physician to suspect a mitochondrial etiology.

MRI in the acute phase demonstrated large asymmetrical confluent lesions simulating acute disseminated encephalomyelitis or tumefactive MS. The lesions most often involved the frontal and parietal region and the periventricular and deep white matter and corpus callosum compared to subcortical or juxta cortical regions and thalamus in children with acquired demyelinating disorders. Contrast enhancement, diffusion restriction, presence of lactate peak and white matter cysts were consistently seen in mitochondrial LE. The evidence of inflammation on MRI is one of the important defining feature of mitochondrial leukoencephalopathies (Kevelam et al., 2016). Restricted diffusion without contrast enhancement is most often seen in ischemia and is attributed to cytotoxic edema. Delayed restricted diffusion with contrast enhancement has been described in tumefactive MS lesions (Hyland et al., 2013). In lesions with restricted diffusion, presence of gadolinium enhancement has been used as a differentiating feature between acute

demyelinating lesions and ischemia (Balashov et al., 2011). This has been attributed to intramyelinic edema or myelin vacuolation as in toxic demyelination or inborn error of metabolism (Sener, 2004). Another alternative explanation is that the myelin breakdown may reduce the water movement in the extracellular space because of the reduced fiber tract organization (Abou Zeid et al., 2012). The presence of concomitant contrast enhancement along with restricted diffusion in mitochondrial leukoencephalopathies may suggest that the pathology is similar to acute demyelinating lesions.

The therapeutic implications of these findings in mitochondrial disorders have not been fully explored. The most important being the utility of glucocorticoid administration in acute stages, as in acute demyelinating disorders. Steroid responsiveness in patients with mitochondrial leukoencephalopathy has been described in patients with LYRM mutations and DARS associated leukoencephalopathy (Dallabona et al., 2016; Wolf et al., 2015). Remarkable corticosteroid response and dependence have been described in patients with MELAS (Gubbay et al., 1989). The similarity in clinical presentation and overlap with primary demyelinating disorder such as MS is already emphasized in literature (Kovacs et al., 2005; Matthews et al., 2015; Weisfeld-Adams et al., 2015). In the sole autopsy study available in patients with LHON-MS, the inflammatory responses are highlighted in detail (Kovacs et al., 2005). Introduction of corticosteroids in this patient intermittently did improve the visual and neurological dysfunction suggesting an early immunological mechanism. It was proposed that mitochondrial dysfunction might occasionally aggravate or initiate the autoimmune process (Kovacs et al., 2005). It remains to be seen if maintenance of the steroid therapy as in other immune mediated disorders can keep a stable course in children with mitochondrial LE. The report of the relapsing remitting MS-like illness in a child with NDUFA1 variation may support this hypothesis. The patient received steroids for achieving as well as maintaining remission. After the remission is maintained, the patient remained clinically stable on mitochondrial cocktail medications. As suggested in the study by Kowacs et al. (Kovacs et al., 2005) the mitochondrial dysfunction in this patient might have initiated the immune response, which got stabilized by the use of steroids. Various mechanisms have been postulated by which glucocorticoids exert its effect on mitochondria (Lee et al., 2013; Psarra and Sekeris, 2011; Tiao et al., 2011).

In conclusion, this study highlights that episodic neuroinflammation is a feature of mitochondrial leukoencephalopathies as evidenced by the clinical presentation and MRI features. These features may overlap with acquired demyelinating disorders. The role of glucocorticoids in inducing and maintaining remission during the neurological episodes in patients with mitochondrial leukoencephalopathy needs to be explored further in prospective studies.

Author contributions

ABT- concept and design, acquisition, analysis and interpretation of data PSB- concept and design, acquisition, analysis and interpretation of data, performed the literature search and wrote the manuscript. ABT, PSB, MN, SS, SC, KS and CV represent the clinical team involved in the evaluation, management and follow up of the patients. HRA carried out the acquisition and interpretation of the radiological data. NG and AM were involved in the acquisition and interpretation of histopathological data. MMSB, JP and KS were involved in the acquisition and interpretation of respiratory chain assays. SC and PG contributed to the interpretation of genetic data. All authors reviewed and approved the final manuscript

Declaration of conflicting interests

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Ethical approval

This study was approved by the Institutional Ethics Committee [No. NIMHANS/91st/2014]

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.msard.2018.01.003.

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