

Clinical and Paraclinical Aspects of Mitochondrial Diseases in 257 Lebanese Children

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Abstract

This is the first retrospective descriptive study, describing the clinical and paraclinical aspects of 257 Lebanese children presenting a mitochondrial disease. The patients were diagnosed over 7 years, with a suspicion of mitochondrial disorder confirmed by muscle biopsy. The clinical and paraclinical signs were studied in this population.

Hypotonia (51.3%), psychomotor delay (11.6%) and epilepsy (7.39%) were the most common presenting signs. With the progression of the disease, hypotonia was found in 80.9% of the patients, while 78.21% reached a condition with multiple disabilities.

The brain imaging showed different lesions in different patients ranging from white matter lesions to basal ganglia lesions and cerebellar lesions. The most common finding on the muscle biopsy was a cytochrome C oxidase deficiency (52.1%) and red ragged fibers were found in 17.5% of the patients. 36.19% of the patients needed at least one time admission to the intensive care unit.

Conclusion: this is the first large scale study on metabolic disorders in Lebanon, it shows that the mitochondrial disorders are the most common metabolic disorders in Lebanon, with a huge variety of presenting signs and symptoms, and with a severe neurological deterioration accompanying the disease progression.

Introduction

Mitochondrial disorders are one of the most common forms of the neurometabolic disorders. The clinical presentation varies greatly in severity and in prognosis and with the essential role of the oxidative phosphorylation all the body organs will be affected. The classical presentation may vary from a pure isolated myopathy to a multisystemic disease with cardiac, renal hepatic and neurological symptomatology.

The incidence of mitochondrial diseases is estimated by 15-10 cases per 100 000 person [1-3] and one study even showed an incidence of 1/16 000 birth per year, with a high prevalence within families of Lebanese descent [4]. The diagnosis is based mainly on a single or combined enzymatic deficiency of the mitochondrial respiratory chain complexes. In the pediatric population the genetic mutations were rarely found with a rate of 5% mutations in 118 patients by Bernier et al. before the whole exome sequencing era [5] and now reaching 16.4% in the new series [6].

The aim of this study is to describe the clinical and paraclinical aspects of the mitochondrial diseases in the Lebanese population, where this is a disorder of a high prevalence, yet with no previously published data.

Material and Methods

Here we present a retrospective study of 257 Lebanese patients, between 0 and 15 years of age, followed at the Saint George University Medical center in Beirut, between January 2010 and December 2016. The diagnosis of mitochondrial disease was suggested following the criteria described by Morava in 2006 [7] and the diagnosis was confirmed in all the patients with a muscle biopsy.

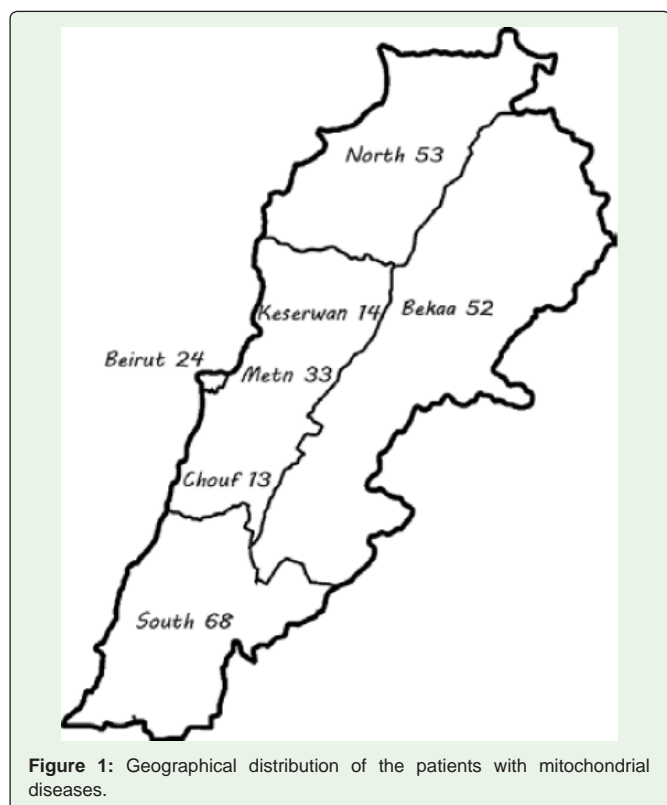


Figure 1: Geographical distribution of the patients with mitochondrial diseases.

The muscle biopsy was studied on paraffin sections stained with H&E and Masson's trichrome. Frozen sections werestained with H&E, modified Gomori's trichrome, PAS+/-D, ORO, ATPase at pH 9.4 and 4.3, NADH-TR, SDH, COX, SDH/COX, NADH/COX; and antibodies to C5b9, Dystroglycans, Laminin, Merosin and ATPsynthase.

The geographical distribution, the parental consanguinity, the family history, the sex, age of first clinical signs, clinical progression as well as the quality of life were studied. Brain MRI with spectroscopy was performed in all the patients, as well as a study of biological markers (lactate, pyruvate, chromatography of amino acids in blood

and organic acids in urine, carnitine blood levels and coenzyme Q 10 blood levels).

Results

257 patients (137 males, 120 females) with mitochondrial diseases were studied, the geographical distribution of this population was homogenous between the Lebanese areas (Figure 1), and we had a homogenous distribution between Lebanese religions (41.6 % Christians, and 58.3% Muslims).

Parental consanguinity was noted in 68.1% of the families and 56% of the families had a positive family history of patients with high suspicion of mitochondrial disease yet without a confirmed diagnosis in most of the cases, and 68 patients presented with neonatal distress, in varied degrees.

The median age for the first clinical complaints was at 10 months of age, and 70% of the patients had a confirmed diagnosis by the age of 4 years.

The clinical presenting signs were very heterogeneous. The most common presenting sign was hypotonia (51.36%), followed by psychomotor delay (11.67%) and then epilepsy (7.39%) (Table 1).

During the course of the disease 30% of the patients presented with epilepsy that was generalized in 16.3%, partial in 3.89%, myoclonic in 2.72%, West syndrome in 3.89% and Ohtahara syndrome in 2.72% of the cases. The west and Ohtahara forms showed a very good response to a ketogenic diet in the first form and to intravenous immunoglobulin in the latter form.

Within the course of the disease 80.9% of the patients showed clear signs of hypotonia and 45.14% showed signs of spasticity, 8.95% became ataxic and 3.89% dystonic.

Cardiomyopathy was found in 11 patients, while 20 patients had a microcephaly. With age progression, 21.01% of the patients showed clear psychomotor regression and 73.15% had a psychomotor delay of varying degrees. Failure to thrive was noted in 54.09% of the patients.

Ninety-three of these patients needed at least one time admission to an intensive care unit.

Table 1: Clinical presenting signs in Lebanese patients with mitochondrial diseases.

	Clinical presenting signs in patients with mitochondrial diseases (n)		(%)
Hypotonia	132		51.36
Psychomotor delay	30		11.67
Epilepsy	19		7.39
Acute Neurological distress	18		7
failure to Thrive	16		6.23
Abnormal movements	14		5.45
regressive Myopathy	9		3.5
liver failure	5		1.95
Gait Disturbance	4		1.56
Cardiomyopathy	4		1.56
arthrogryposis	3		1.17
anemia	3		1.17

Table 2: Biological markers finding in Lebanese patients with mitochondrial diseases.

	Biological markers	(n)	(%)
Chromatography of amino Acids in blood	Normal	210	81.71
	Glycine increase	7	2.72
	Alanine increase	12	4.67
	Increase of glu/gln pool	13	5.06
Chromatography of Organic Acids in urine	Normal	207	80.54
	Methyl Malonic Acid in urine	13	5.06
	Krebs cycle products	23	8.95
	Lactic Acid	53	20.62
Decreased Coenzyme Q10 blood level		15	5.84
Decreased Carnitine Blood level		27	10.51

Regarding the biological parameters, 40% of the patients had an increase in the arterial blood levels of lactic acid and pyruvic acid. In the chromatography of amino acids in blood glycine, alanine and glutamine were found to be increased in some of the patients, while in the urine organic acids chromatography lactic acid was found in 20.6% of the patients and krebs cycle products in 8.95% of the patients. Carnitine was decreased in 5.84% and co-enzyme Q 10 in 10.51% (Table 2). Seventeen patients had CPK increase, 2 of them with a level reaching 1000 IU/L during episodes of sickness.

Brain MRI and spectroscopy was performed in all the patients, and it showed a thin corpus callosum in 70 patients, cerebral atrophy in 57 patients, leukodystrophy in 50 patients (Figure 2) and basal ganglia lesions in 25 patients (Figure 3). A Leigh/MELAS [8] radiological pattern was found in 10 of the patients. On the Spectroscopy a lactate peak was identified in 7 of the patients (Figure 4).

The pathological studies showed global mitochondrial dysfunction in 19% of the patients, muscle fibers 1 hypertrophy in 50% of the patients, as well as glycogen overload, red ragged fibers were found in 9.72 % of the patients, and associated neurogenic dysfunction was noted in 15.5% of the patients. The most common complex deficiency was in the complex IV of the respiratory chain (Table 3 and 4).

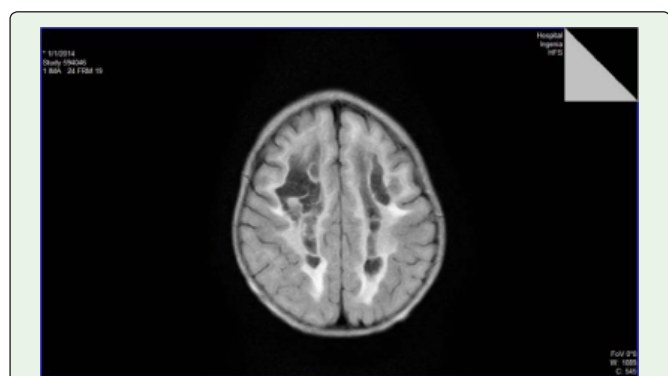


Figure 2: Brain MRI showing diffuse white matter abnormal signal in a patient with mitochondrial myopathy with Complex II and complex V deficiency.



Figure 3: Brain MRI showing basal ganglia lesions with subcortical lesions in a patient with mitochondrial myopathy with Complex IV deficiency.

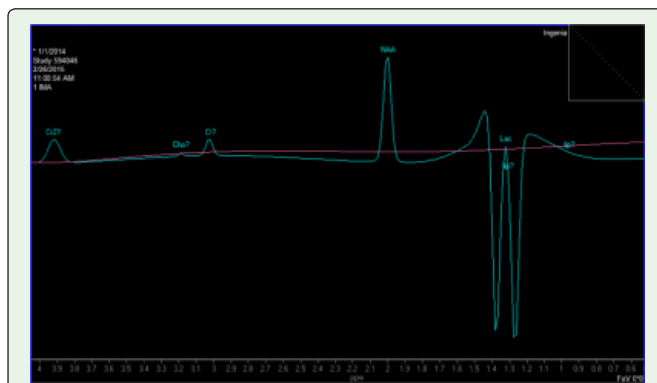


Figure 4: Brain MR Spectroscopy showing increase in N-acetylaspartate and lactate peak in a patient with mitochondrial myopathy with Complex II and complex V deficiency.

Table 3: Radiological findings on Brain Magnetic resonance and spectroscopy in Lebanese patients with mitochondrial diseases.

	MRI and MRS results	n	%
Corpus Callosum	Normal	183	71.21
	Thin	70	27.24
	partial agenesis	4	1.56
			0.39
Cerebral Atrophy		57	22.18
Cortical Lesions		21	8.17
Leukodystrophy		50	19.46
Basal Ganglia Lesions		25	9.73
Cerebellar Atrophy		6	2.33

Genetic studies were not accessible to all the patients due to the excessive cost.

All the patients in this series were entitled to the national card for the disabled and were followed in rehabilitation centers with physical therapy and speech therapy and special schooling.

Table 4: Muscle Biopsy results in Lebanese patients with mitochondrial diseases.

Muscle Biopsy Results	%
CI: NADH dehydrogenase	30.1
CII: succinate dehydrogenase	48.2
CIII: cytochrome bc1 complex	23.3
CIV: cytochrome c oxidase	52.1
CV: ATP synthase	38.1
Global mitochondrial dysfunction	19
Hypertrophy Muscle Fibers I	50.01
Glycogen overload	50.01
Lipids overload	56.4
Red Ragged Fibers	9.72
Presence of mitochondrial aggregates	32.2
Associated neurogenic dysfunction	15.5

Discussion

Lebanon is a Mediterranean country with a population of 6,000,000 inhabitants, living in a 10,452 km² area. The population consists of 18 founder religious groups, with a very high rate of consanguinity which led to the increase of the incidence of multiple rare diseases in the same patient [9,10]. No national statistical data are available in Lebanon, the only published data comes from one medical center and it shows the predominance of mitochondrial diseases as the most common metabolic disease in Lebanon [11]. After the civil war the Lebanese diaspora population increased in the 5 continents and kept the high rate of consanguineous marriages, a founder effect for a mitochondrial disease mutation PET 100 was found in a big series of patients with mitochondrial diseases who were of Lebanese descent in Australia [12].

Here we present the first and largest series of patients with mitochondrial disorders in Lebanon, with 257 patients followed over 7 years who fulfill Morava's criteria for mitochondrial diseases [13].

For this disease, the first signs of energy deficiency are noted in Utero. In our series 27.2 % of the patients have a thin corpus callosum on the MRI which is a sign of prenatal signs of biological distress.

Most of our patients were diagnosed at early age. The neonatal forms have a very severe prognosis as reported by Garcia-Carzola et al [14]. with a severe encephalopathy in 97.4% of the cases and 30.7% of cardiomyopathy. The prevalence of cardiomyopathy differs following the series [15]. In our series we did not find many patients with cardiomyopathy. This can be due to death before referral from peripheral hospitals, causing the under diagnoses of this entity.

In mitochondrial diseases, the most common signs are of a neurological nature in a rate varying from 45% of the cases [16] to 90.3% in more recent series.

This is mainly due to disruption of mitochondrial biogenesis, turnover and functions, which can even contribute to different phenotypes in some neurodevelopmental and neurodegenerative

diseases. In our series 50% of the patients presented with hypotonia followed by psychomotor delay, epilepsy and acute neurological distress. The increase in the neurological presentation can be the result of the late referral due to the patients' low economic status in the rural areas.

Epileptic forms are usually linked to a complex I deficiency mainly [17]. In our series most of the patients had a complex IV deficiency, yet epilepsy was noted in these patients as well, which can be expected since each complex deficiency may present with many different phenotypes. In our series we noted a very good response to a ketogenic diet in patients with West syndrome and repetitive courses of IVIG were very beneficial in controlling the seizures in patients with Ohtahara epilepsy.

On the MRI we found variable degrees of static or progressive lesions and we noted many patients having severe white matter lesions upon the initial distress episode yet with an acceptable motor and neurocognitive progression. A genetic study of the mutation would help clear the physiopathology in these patients. Many patients had a Leighor Leigh/MELAS radiological presentation, which is a classical feature of mitochondrial diseases [18].

Oligodendrocytes and myelin production are very sensitive to mitochondrial dysfunction [19]. When affected they promote a demyelination process that can be seen as an increase in the choline peak on MR spectroscopy [20]. These MR spectroscopy findings help evaluate the severity of the disease, without being specific for the mitochondrial diseases. Cerebellar atrophy was noted in 6 patients. This is due to the fact that the cerebellar neurons are very susceptible to oxidative stress [21].

Ophthalmological signs in mitochondrial diseases are very rare in pediatrics and mainly described in adult forms, mostly strabismus which can manifest during childhood [22].

Lactic acid elevation was noted in 40% of the patients, but this is a reduced number from an initial higher value where errors of sampling were committed.

Alanine and glutamine levels were found increased in a combined value of 10% of the cases, less than reported in literature [14]. The variability of the biological markers shows the necessity of the muscle biopsy which remains a key element for diagnosis, especially when there is no availability of genetic studies. Typical lesions like the red ragged fibers [23] are not as common in children as in adults [24], yet it was found in 25 of our patients. The most common finding would be increases in glycogen and lipids, yet a normal biopsy does not exclude the diagnosis [25].

Our study is a retrospective descriptive study aiming to assess the current status of mitochondrial disorders in Lebanon. A major limitation is the absence of biochemical measurement of the complexes of the respiratory chain on the muscle biopsy which is a technique that has been introduced recently to Lebanon. And a further barrier is the paucity of genetic studies in mitochondrial patients because of the high cost of the exams and the absence of a third party payer covering genetic investigations [26-28]. Another major fact limiting the rush towards the genetic investigations is that Lebanon remains a religious community by excellence with limited ethical options in prenatal diagnosis, as well as the illegality of pregnancy termination.

Conclusion

Mitochondrial diseases are the most common cause of inborn error of metabolism in Lebanon. The frequency remains under estimated, and most of the patients remain under diagnosed. Our numbers are higher than reported in literature due to the high rate of consanguinity. The clinical presentation is very diverse, with a wide spectrum of biological markers changes as well as typical radiological signs, but the main diagnostic procedure remains the muscle biopsy study. Here we presented the first Lebanese series of patients with mitochondrial diseases. Further investigations and more accurate techniques must be considered in the future. A nationwide awareness program about the risks of consanguinity should be initiated and a national registry for the inborn errors of metabolism should be established, in order to offer the best follow up for the patients.

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