Clinical and Biochemical Aspects of Mitochondrial Disorders in Egyptian Patients

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ABSTRACT

Mitochondrial disorders are a heterogeneous group of disorders caused by defects in intracellular energy production. They are characterized by morphological and biochemical abnormalities of mitochondria. In this study, the pathological finding of ragged red fibers in the muscles verified the diagnosis of mitochondrial disorders in 19 patients. All the patients were subjected to full clinical examination, biochemical examination including plasma lactate, plasma pyruvate, lactate/pyruvate ratio and plasma cytochrome C. Neurophysiological examination including electroencephalogram (EEG), electromyelogram (EMG), echocardiogram (Echo), ultrasonography (US), electroretinogram (ERG), hearing test and magnetic resonance imaging (MRI) were done. The aim of the work was to review the clinical, neurophysiological findings in Egyptian patients with mitochondrial disorders. To emphasis the important findings, which let us suspect that we are dealing with a mitochondrial patient. To annotate the debate about apoptosis in mitochondrial disorders. In conclusion the features increasing the likelihood of mitochondrial disorders were: (i) atypical phenotype, (ii) maternal inheritance pattern and (iii) Ragged red fibers in the muscle biopsy, (IV) neurophysiological impairment, (V) increased lactate, pyruvate, lactate/pyruvate ratios and cytochrome C in plasma. Mitochondria have also been found to play a central role in programmed cell death (apoptosis). Efficient laboratory diagnosis of mtDNA is of great importance for the accurate diagnosis of mitochondrial disorders. **(Int. J. Ch. Neuropsychiatry, 2004, 1(1): 41-50)**

INTRODUCTION

Mitochondrial disorders represent an expanding clinically heterogeneous disorder caused by defects in intracellular energy production and are generally associated with mitochondrial (mtDNA) mutations or nuclear gene defect[1]. Clinical symptoms originating from all organ systems have been described, but tissues with high-energy requirements such as muscles and brain are particularly vulnerable[2]. However, multisystem disorders and non-specific neurological symptoms are common in children[3].

The complexity of mitochondrial metabolism, its central role in energy production, its dual genetic control (mitochondrial and nuclear DNA) may explain the exceptional heterogeneity of mitochondrial disorders[4]. However, mtDNA mutations reduce the function of the respiratory chain, leading to increased production of reactive oxygen species (ROS), which can attack the mitochondrial membrane. This may lead to leakage of mitochondrial compounds such as cytochrome C and apoptosis inducing factor (AIF) and the co-enzyme Q10. The

respiratory chain component CoQ10 is believed to counteract mitochondrial permeability transition pore (mPTP) opening, which is the main trigger of the cell suicide[5]. The ROS can also cause mt DNA mutations, which aggravate the respiratory chain function. The impaired oxidation will lead to reduced ATP production and thus also cause difficulties in maintaining the transmembrane potential[6].

Noteworthy, other mechanisms are also implicated, such as incorrect assembly of the defective enzyme complex or complexes of other mitochondrial proteins, the production of free radicals and apoptosis may be postulated to explain the phenotypic variability and the severity of mitochondrail diseases[4]. Apoptosis is an essential mechanism for tissue development and homeostasis. It requires the activation of specific genes that lead to a series of distinctive morphological and biochemical features[7]. In human muscle pathology, apoptotic nuclei and apoptosis-related proteins, AIF, have been demonstrated in atrophic fibers in neurogenic muscle atrophy[8].

Several distinct syndromes have been recognized among the variable clinical phenotypes of mitochondrial disorders including: chronic progressive external ophthalmoplegia, Kearns Sayre syndrome (KSS), mitochondrial encephalopathy with lactic acidosis and stroke like episodes (MELAS), myoclonus epilepsy with ragged red fibers and neurogenic muscle weakness with neuropathy ataxia and retinitis pigmentosa[9]. KSS and MELAS show characteristic lesions on computed tomography (CT) and magnetic resonance imaging (MRI) studies[10,11] and typical imaging findings are the hallmark of diagnosis of Leigh syndrome[12,13]. Neuroimaging has proved useful in the diagnosis of mitochondrial disorders.

The aim of the work was to review the clinical, neurophysiological findings in Egyptian patients with mitochondrial disorders. To emphasis the important findings, which let us suspect that we are dealing with a mitochondrial patient. To annotate the debate about apoptosis in mitochondrial disorders.

MATERIALS AND METHODS

The study included 19 patients suspected to have mitochondrial disorders. They were referred to the Pediatric Department, El-Hussein Hospital. Their ages ranged from 2 years to 13 years. Fifteen healthy age and sex matched children served as controls.

Each patient was subjected to full personal and medical history, meticulous clinical examination to detect any malformation or anomaly. Three generations pedigree were constructed and analyzed with special emphasis on maternal inheritance, positive parental consanguinity and similarly affected members in the family.

In addition, the following investigations were done to all patients:

1- *Neurophysiological investigations:*
   - A- Electroencephalogram (EEG).
   - B- Electromyelogram (EMG).
   - C- Echocardiogram (Echo).
   - D- Ultrasonography (US).
   - E- Complete eye evaluation including electroretinogram (ERG)
   - F- Hearing test.
   - G- Magnetic resonance imaging (MRI).

2- *Biochemical assessments:*
   - A- Plasma lactate[14].

B- Plasma pyruvate[15].
C- Lactate/pyruvate ratio.
D- Plasma cytochrome C was done by ELISA technique according to Wetzel et al.[16] using commercial kit provided by Medicopharma trade diagnostics, fine chemicals and pharmaceutical chemicals suppliers.

The biochemical data were statistically analyzed using the spss package software.

3- *Pathological examination:* Muscle biopsy stained by the modified Gomori trichrome stain[17].

RESULTS

A total of 19 patients were examined, their ages at referral ranged from 2 years to 13 years. The age of onset ranged from 6 months to 7 years. The ratio of males to females in the studied patients was 8:11 (.73).

History and pedigree analysis revealed positive consanguinity in 47.4% of the studied families (Fig.1: a and b) showing two pedigrees with maternal inheritance. Mitochondrial disorders are inherited almost exclusively from mother to children as illustrated in these pedigrees.

Pregnancy and delivery histories were irrelevant in all patients.

Clinical examination of patients (Table 1) revealed delayed milestones in 10 patients (52.6%), 10 patients showed facial dysmorphism in the form of hypertelorism, epicanthal folds, depressed nasal bridge, bossing of forehead and long philtrum.

Neurological abnormalities were present in 18 patients (94.7%), hypotonia in 12 patients (63.1%) and hypertonia in 5 patients (26.3%). Seizures and mental retardation were present in 10 patients (52.6%). Ataxia was present in 2 patients (10.5%).

Table (2) shows the neurophysiological findings of electroencephalogram (EEG) in 10 patients, showed generalized epileptogenic dysfunction. Electromyelogram (EMG) in 9 patients showed myopathic changes with proximal muscles more involved than distal muscles. Echocardiography (ECHO) in 2 patients showed cardiomyopathy. The ultrasonography in 4 patients showed hepatomegaly. The electroretinogram (ERG) in 6 patients showed retinitis pigmentosa. The hearing test in 4 patients showed severe sensorineural hearing loss in 2 patients and moderate sensorineural hearing loss in the other 2 patients. The MRI in 16 patients showed abnormal signal affecting both grey and subcortical white matter. Generalized cerebral atrophy was frequent (Fig. 2: a and b).

The muscle biopsy of all patients showed ragged red fibers on modified Gomori trichrome stain, which proved that we are dealing with a mitochondrial disorder (Fig. 3: a and b).

Table (3) illustrates the mean and standard error of plasma lactate, plasma pyruvate, lactate/pyruvate ratio and cytochrome C among the studied group and controls. The table shows significantly higher levels of all the biochemical parameters in our patients than controls p= 0.000.

The percentage of change in biochemical parameters were: 115, 100, 34, 370 for plasma lactate, pyruvate, lactate/pyruvate ratio and cytochrome C respectively.

Table 1. Clinical findings.

Serial no	sex	Age of onset	F.H.[1]	Cons[2]	Dev. Delay[3]	Dysmorphism	Neuro[4]	Fundus	Hearing test	Organomegaly
1	F	3y	+	+	+		+	+		+
2	M	2.5y					+	+	+	
3	F	1y		+		+	+	+		
4	F	6m			+	+	+			+
5	F	1.5y	+				+	+		
6	M	6m			+	+	+		+	
7	M	8m	+	+		+	+		+	
8	F	4y			+		+	+		
9	M	2y		+	+		+	+		
10	M	1y				+	+			
11	M	9m	+	+	+	+	+			
12	F	5y	+					+	+	
13	M	7y		+	+		+			+
14	F	1.5y	+	+		+	+	+		
15	F	1y			+	+	+			
16	F	3y		+			+	+		
17	F	6m			+	+	+	+		+
18	M	2y	+	+			+			
19	F	3y			+	+	+	+		

1: Family history. 2: Consanguinity. 3: Developmental delay. 4: Neurological findings.

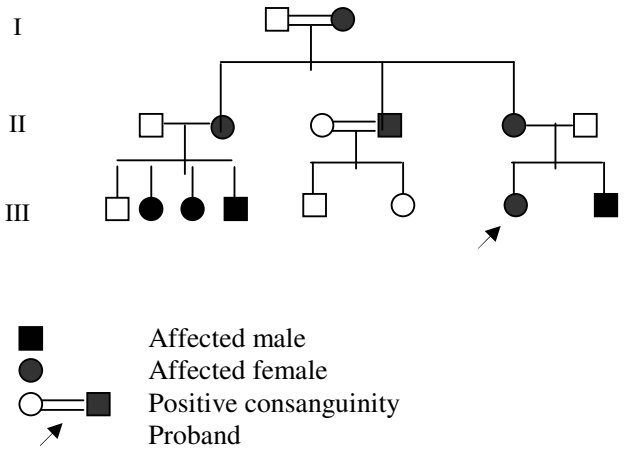
Table 2. Neurophysiological findings.

Serial No.	EEG[1]	EMG[2]	Echo[3]	US[4]	ERG[5]	Hearing test	MRI[6]
1	+			+	+		+
2	+	+				+	+
3					+		+
4	+	+		+			+
5		+					
6	+	+				+	+
7	+	+				+	+
8			+		+		+
9		+					+
10	+						+
11	+						+
12		+			+	+	
13	+			+			+
14							+
15	+						+
16			+		+		+
17		+		+			
18	+						+
19		+			+		+

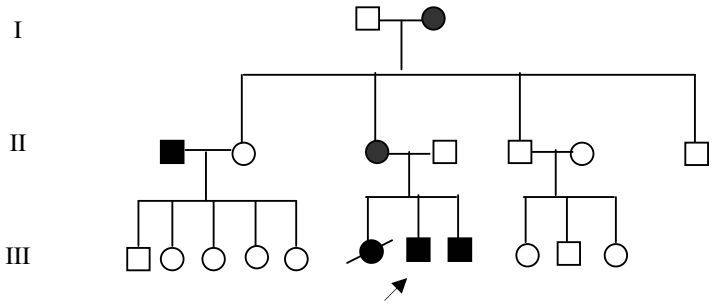
1: Electroencephalogram. 2: Electromyelogram. 3: Echocardiogram.
4: Abdominal sonography. 5: Electroretinogram. 6: Magnetic resonance imaging of the brain.

Table 3. Biochemical parameters in patients with mitochondrial disorders (mean ±S.E).

Biochemical parameters	Patient n=19	Control n=15	P value
Plasma lactate mg/dl	28.4 ± 0.99	13.2 ± 0.65	0.000
Plasma pyruvate mg/dl	1.6 ± 0.05	0.8 ± 0.02	0.000
Lactate/pyruvate ratio	20.4 ± 0.73	15.2 ± 0.67	0.000
Plasma Cytochrome C ng/ml	187.1 ± 5.02	39.8 ± 0.9	0.000



(a): Pedigree of case number 4.



(b): Pedigree of case number 7.

Fig. (1 a and b): Showing two pedigrees with maternal inheritance.

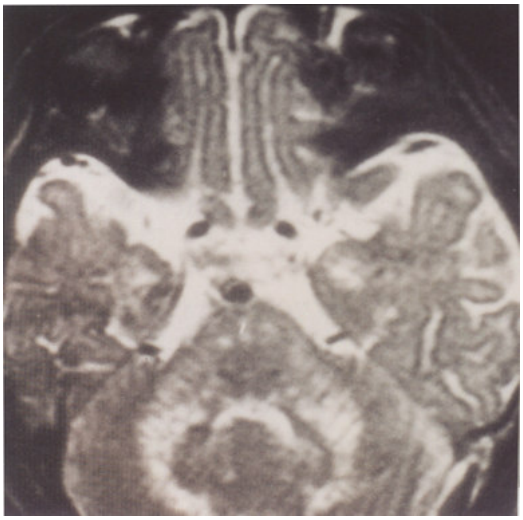


Fig. (2a): MRI of the brain of case No 7 showing demyelination of cerebellum.

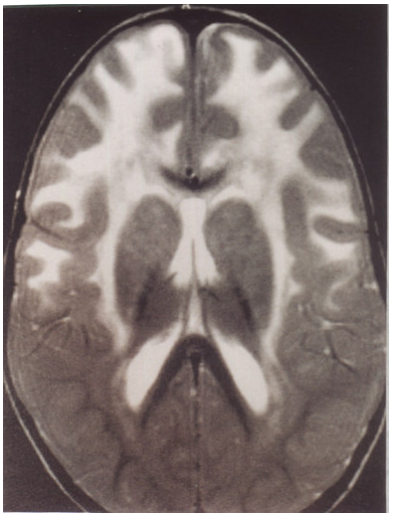


Fig. (2b): MRI of the brain of case No.2 showing T_2 hyper intensity and demyelination involving fronto-parietal areas.

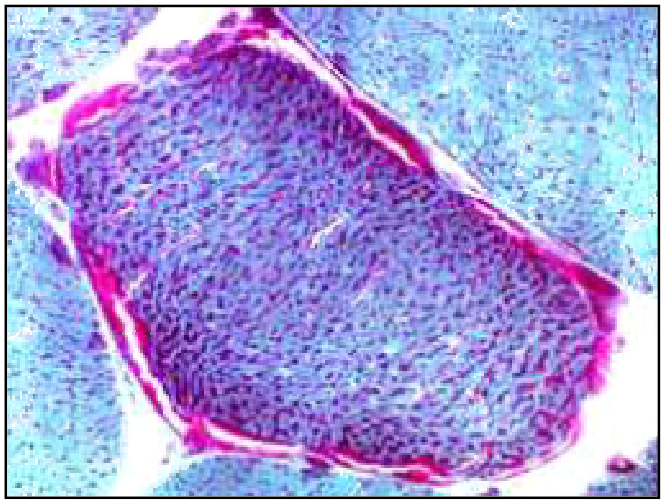


Fig. (3a): Muscle fibers showing mild mitochondrial proliferation (red rim). Gomori trichrome stain x 900.

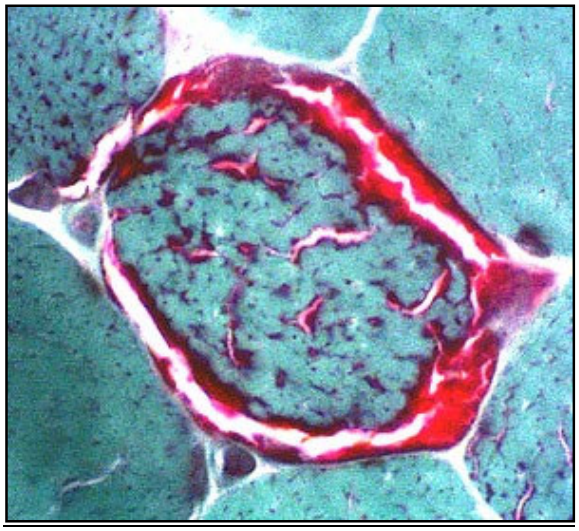


Fig. (3b): Muscle fibers showing moderate mitochondrial proliferation. Gomori trichrome stain x 940.

DISCUSSION

The mitochondrial disorders are a clinically diverse group of disorders defined by the presence of structural or functional abnormalities in mitochondria. Muscle biopsy plays an integral role in evaluation of the patients with mitochondrial disorders. It is an absolute essential part of the diagnostic investigation. In the current study, ragged red fibers (RRF) in the muscle biopsy verified the diagnosis of mitochondrial disorders in 19 patients. This coincides with the findings of Fadic and Johns[18] who mentioned that ragged red fibers in skeletal muscle biopsy were regarded as the histological marker of mitochondrial disorders. Valanne et al.[2], mentioned that the diagnosis of mitochondrial disorders is based on the presence of clusters of mitochondria in muscle cells (ragged red fibers). However, Mandava emphasized that although the presence of RRF in muscle biopsy specimen is a strong indication of a mitochondrial disorder, a negative muscle biopsy do not preclude the consideration of mitochondrial disorders[19].

The male to female ratio is 8:11, which is different than Gozalbo et al. who found male preponderance among his patients; however the severity of clinical manifestations was similar in both sexes[20]. Positive consanguinity was present in 47.4% of our patients and 36.8% had similarly affected family members. Mitochondrial disorders are inherited almost exclusively from mother to children as illustrated in the pedigrees. Fadic and Johns mentioned that during fertilization, all the mt DNA is derived from the oocyte[18]. Therefore, genetic

diseases arising from mt DNA mutations are maternally inherited, i.e. a mother carrying a mt mutation will transmit it to all her children, but only her daughters will pass it on to all her children.

Mitochondrial disorders are clinically and genetically heterogeneous because mitochondria are the products of 2 genomes: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). Hirano et al, 2004 mentioned that the phenotype depends on the number of normal and mutant mitochondria in each cell. Thus the mitochondrial disorders are characterized by atypical phenotype.

Delayed milestones, which is usually the initial manifestation, was present in 10 patients (52.6%). This coincides with the findings of Fadic and Johns[18]. Garcia et al. also described delayed mile stones in their patients which were one of the earliest signs to appear[21].

Facial dysmorphism was present in 10 patients (52.6%) in the form of frontal bossing, depressed nasal bridge, hypertelorism and recession of anterior hairline. Short stature was present in 5 patients. Shanske and Di Mauro[22] and Finsterer[23] described the presence of short stature in their mitochondrial disorder patients also, 4 patients were underweight which coincides with the findings of Fadic and Johns[18].

Mental retardation and seizures were detected in 10 patients (52.6%), with an IQ below 65. This coincides with the findings of Melone et al. who found mental subnormality and severe myoclonic seizures in their mitochondrial patients[24].

The EEG changes encountered in our patients were focal epileptogenic abnormalities with atypical spikes and waves. This is in agreement to the findings of Fadic and Johns[18]. Ataxia was present in 2 patients, hypotonia in 12 patients (63.1%) which coincides with the findings of Gozalbo et al[20].The EMG showed myopathy in 9 patients (47.4%), Fadic and Johns mentioned that myopathy may be very mild clinically and can be only detected by EMG.

Eye evaluation showed ptosis in 3 patients, nystagmus in 2 patients and cataract in 2 patients. ERG showed retinitis pigmentosa in 6 other patients. Biousse and Newman,[25] said that Neuro-ophthalmic signs figure prominently and may be the presenting or even sole manifestation of these disorders. The four most common neuro-ophthalmic abnormalities seen in their patients were bilateral optic neuropathy, ophthalmoplegia with ptosis, pigmentary retinopathy, and retrochiasmal visual loss.

Sensorineural hearing loss was present in 4 patients (21.1%) it is progressive to the point that amplification devices were indicated. Ceranic and Luxon[26] described bilateral hearing loss in their patients.

Cardiovascular manifestations were detected in 2 patients (10.5%). Echocardiogram showed cardiomyopathy. Similarly, Liao et al.,[27] described hypertrophic cardiomyopathy and echocardiogram changes were found in 42.8% of their patients.

There was hepatomegaly in 4 patients (21.1%) similar results were described by Finsterer[23].

Abnormal MRI was present in 16 patients (84.2%) T_2 hyperintensity of cerebral white matter and cerebellar atrophy in 12 (63.2%) and basal ganglia calcification in 2 (10.5%). This coincides with the findings of Hirano et al.[28], and Parry and Matthews[29] who emphasized that MRI is important in the diagnosis of mitochondrial disorders for they

offer important clinical tools for patient assessment.

The biochemical parameters showed significant increase in lactate, pyruvate and lactate/pyruvate ratio in plasma of all patients. Fadic and Johns[18] mentioned that determination of lactate and pyruvate levels may serve as a simple metabolic screening test for mitochondrial disorders for their increase is a marker of mitochondrial dysfunction. The increase in lactate/pyruvate ratio is a sign of respiratory chain defect[19]. Cytochrome C is a soluble protein loosely attached to the inner mitochondrial membrane; it is an essential component of the mitochondrial respiratory chain and plays an important role in apoptosis Mirabella et al.[4]. Apoptosis (programmed cell death) is essential in tissue development and homeostasis and plays a role in pathogenesis of mitochondrial disorders. Alteration of apoptosis triggers malformations. Cytochrome C was increased in all our patients. This coincides with the findings of Mirabella et al.[4], who suggested that apoptosis is not simply a means whereby cells with dysfunctional mitochondria are eliminated, but that it seems to play a role in the pathogenesis of mitochondrial disorders associated with mt DNA defects. The imbalance and relative abundance of nuclear encoded and mt DNA encoded subunits may favor cytochrome C inactivation and release. Cytochrome C together with respiratory chain dysfunction could activate apoptotic pathways that in turn inhibit the rate of mitochondrial translation and importation of nuclear encoded mitochondrial protein precursors. This vicious circle may amplify the biochemical defects and tissue damage and contribute to the modulation of clinical features. In mitochondrial disorders, apoptosis represents a selective mechanism for the elimination of cells with dysfunctional mitochondria and excessive free radical production or, may amplify cell damage. Thus cytochrome C, antioxidants and CoQ10 may represent cure for mitochondrial disorders through the specific killing of the cells that accumulate mt DNA.

Features increasing the likelihood of mitochondrial disorders include the following: (i) atypical phenotype, (ii) maternal inheritance pattern and (iii) Ragged red fibers in the muscle biopsy, (IV) neurophysiological impairment, (V) increased lactate, pyruvate, lactate/pyruvate ratios and cytochrome C in plasma. Efficient laboratory diagnosis of mtDNA is of great importance for the accurate diagnosis of mitochondrial disorders.

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