

## Case Report

### Histochemical and Electron Microscopic Diagnosis of Mitochondrial Myopathy: The First Case Report From Iran

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#### ABSTRACT

Muscle tissue, skeletal muscle as well as cardiac muscle, is commonly affected in mitochondrial disorders. One explanation for this observation is that muscle tissue has a high-energy demand and therefore is more sensitive to a deficiency of mitochondrial energy production than some other tissues. In mitochondrial disorders, skeletal muscle tissue may be affected primarily by defective respiratory chain function or secondarily to peripheral neuropathy with neurogenic muscle atrophy. The clinical manifestations of mitochondrial myopathies are variable and include muscle weakness, exercise induced cramps and myalgia. Also, ptosis and progressive external opthalmoplegia are typical but not obligate finding. Hereby we wanted to report a case of mitochondrial myopathy, diagnosed by histochemical and electron microscopic studies for the first time in Iran.

Our case was a 12 year old girl who referred due to muscle weakness to our center which started an 4 years ago. Later, she also developed ptosis. EMG studies were inconclusive and muscle biopsy revealed typical red ragged fibers with special staining. By electron microscopy, typical mitochondrial changes were detected.

**Key words:** Mitochondrial Myopathies, Electron microscopy, Histocytochemistry

#### Introduction

Mitochondria were first observed by Altman 110 years ago and he suggested that they were free living 'elementary organisms' within the cells (1). These organelles are active in oxidative phosphorylation in cells and generation of ATP as energy source (2). The mitochondrial DNA (mtDNA) was discovered in 1963 (3) and the complete sequence for human and mouse mtDNA was available in 1981 (4). Mitochondrial myopathy is the term applied to

a clinically and biochemically heterogeneous group of disorders which have multisystem involvement. The concept was introduced by Luft in 1962 (5). This discovery meant a revolution in the field of mitochondrial medicine. Today we know more than 50 point mutations and 200 different rearrangements of mtDNA that cause mitochondrial dysfunction (2). In addition, several nuclear mutations causing mitochondrial disease have been identified (6). Furthermore several lines of indirect evidence suggest

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that mitochondrial dysfunction may also have a role in common disorders such as heart failure, diabetes mellitus and neurodegeneration (7).

Disorders of oxidative phosphorylation may affect any tissue, and clinical signs and symptoms commonly indicate multiple organ involvement (8). Muscle tissue, skeletal muscle as well as cardiac muscle, is commonly affected in mitochondrial disorders. Muscle tissue is commonly investigated even if there are no major signs or symptoms of myopathy (8). Often there is accumulation of mutant mtDNA in cells before oxidative energy production is impaired, the threshold being more than 90%, this means that a small amount of normal mtDNA can rescue energy production by cells (8).

In mitochondrial disorders, skeletal muscle may be affected primarily by defective respiratory chain function or secondarily to peripheral neuropathy with neurogenic muscle atrophy. The clinical manifestations of mitochondrial myopathies are variable and include muscle weakness, exercise induced cramps and myalgia (9). Typical manifestations of mitochondrial myopathy are ptosis and progressive external ophthalmoplegia (10). In patients with unexplained acquired juvenile unilateral ptosis, an underlying mitochondrial cytopathy should be considered even in cases of inconspicuous ancillary examinations comprising skeletal muscle histology and biochemistry (11). A rare manifestation is episodic weakness associated with rhabdomyolysis and myoglobinuria (10).

Cardiac involvement is common in mitochondrial disorders and may be expressed in various ways such as dilated cardiomyopathy and atrioventricular conduction blocks (12).

Histopathologic analyses of muscle from patients with mitochondrial myopathy usually reveal typical alterations. The hallmark of mitochondrial myopathy is the ragged red fiber (13,14), which is a muscle fiber exhibiting accumulation of mitochondria that is stained red by the modified Gomori trichrome technique (13). The accumulated mitochondria are usually ultrastructurally abnormal with disorganized cristae and often contain paracrystalline inclusions (14,15). Studies of longitudinal muscle sections have demonstrated that red ragged fibers are segmental abnormalities not involving the entire length of the muscle fiber. Different mtDNA mutations may occasionally present with a dystrophic pattern in muscle, but the usual morphological appearance of mitochondrial myopathies does not include dystrophic changes (8). Molecular genetic methods can also be used to detect specific mtDNA and nuclear gene

mutations (8). Due to the extremely variable clinical presentations of these disorders, a complete clinical and laboratory workup involving strict diagnostic criteria is essential (5).

Unfortunately, the recent major advances in understanding of the pathogenesis of mitochondrial disorders has not lead to effective treatment although agents such as coenzyme Q, tocopherol and succinate are currently in use (16). The ultimate treatment would be gene therapy aimed at replacing or repairing the defective gene (8). Due to lack of routine and proper diagnostic methods in the field of hereditary myopathies in Iran, most cases are missed and so we do not have report of cases exists data of prevalence of this disease exists and to our knowledge this is the first report with complete histochemical and electron microscopic data in our country.

## Case report

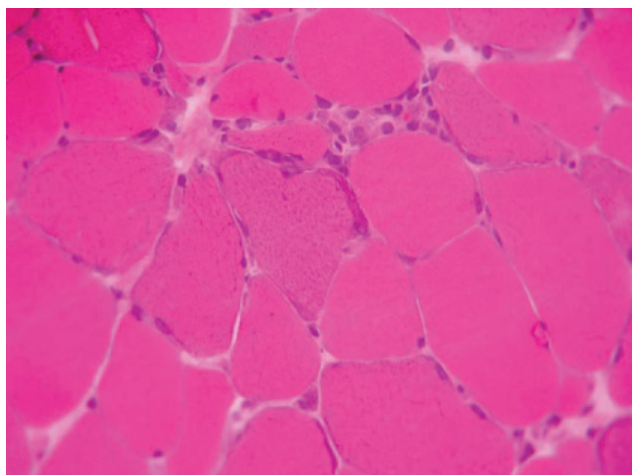
A twelve-year-old female referred due to progressive muscle weakness started since 8 years of age to our center. One year later, she also developed ptosis, but she has no difficulty in swallowing. Physical examination revealed muscle weakness (proximal weakness in upper and lower extremities: force: 4/5) and ptosis, otherwise unremarkable. Family history was negative.

Laboratory data revealed an elevated serum lactate 60 (normal: 4,5-20) in one occasion.

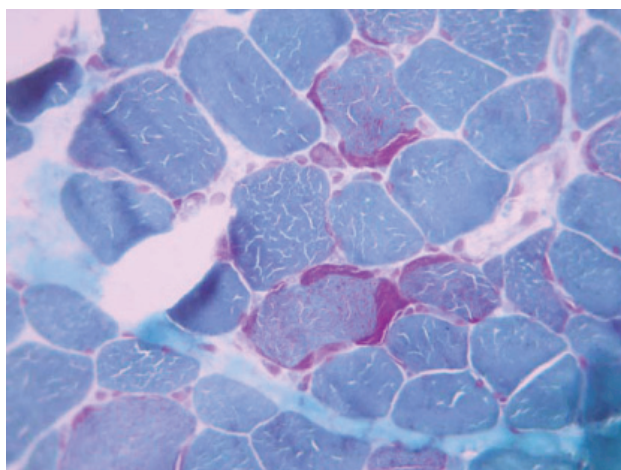
Muscle enzymes were not elevated and electrodiagnostic studies were inconclusive and she was referred for muscle biopsy with strong suspicion of mitochondrial myopathies.

Fresh muscle specimen were obtained from patient and freezed in liquid nitrogen, 3 micrometer sections were prepared by freezing microtome and special staining methods including Gomori trichrome and NADH (nicotinamide adenine dinucleotide hydrogenase) were performed. Also a piece of fresh biopsy was put in glutaraldehyde for electron microscopic studies and grids were prepared by routine technique.

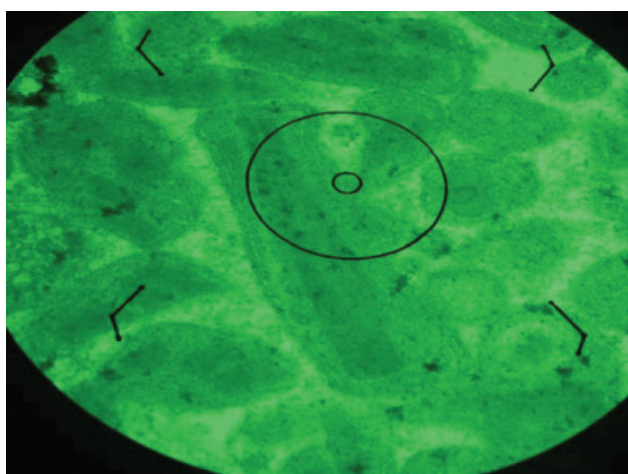
Usual Hematoxylin and Eosin staining revealed typical ragged fibers (Figure 1). By Gomori trichrome stain, the ragged fibers were better demonstrated and clumps of red stain were seen in and around the periphery of the cell, which is due to accumulation of abnormal mitochondria (Figure 2). Electron microscopic studies were done (which are solely performed in our center in clinical settings) and revealed abnormal mitochondria (Figure 3).



**Figure 1 . Hematoxylin and eosin stain of skeletal muscle with typical fibers that appear ragged (H&E  $\times 400$ ).**



**Figure 2. Gomori Trichrome stain reveals better the fibers that are ragged and the mitochondria stain red by this method.**



**Figure 3. Abnormal large mitochondria with distorted cristae in electron microscopic study.**

## Discussion

The first description of a patient with mitochondrial myopathy and deficient respiratory chain function was reported by Luft and coworkers almost 40 years ago (5). Subsequent morphological and biochemical studies in following decades aimed at identifying patients with mitochondrial diseases. These are protein disorders resulting from mutations in both mitochondrial and nuclear genes that are necessary for the biogenesis and function of respiratory chain in mitochondria and cause symptoms in tissues such as central nervous system, heart, skeletal muscle, liver and kidney, which have high-energy demands and are therefore particularly sensitive. In most centers, diagnosis involves clinical evaluation and the morphological, histochemical, and biochemical investigation of skeletal muscle biopsy (14).

Although mitochondrial diseases are rather prevalent in most parts of the world, in a recent epidemiological study carried out in north eastern England, Chinnery et al demonstrated that overall 12.48 per 100000 individuals in the adult and child population either had mtDNA disease or were at risk of developing mtDNA disease (17), while these disorders have been diagnosed since many years ago, but unfortunately diagnostic tools were not developed in our country until recently, although genetic studies of this entity has been instituted. Therefore, this may be the first case of mitochondrial myopathy reported from Iran with rather complete histochemical and electron microscopic studies.

The most prevalent tissue studied in these disorders is skeletal muscle and diagnosis demands study on fresh muscle with histochemical stains mainly by Gomori trichrome stain and also diagnosis is confirmed by electron microscopic study, but unfortunately muscle biopsies are mostly sent in formalin by surgeons which will result in complete missing of the morphologic changes. We hope that with better handling of muscle tissue and specialized studies, we can diagnose these disorders more and more.

Genetic studies are an important part in studying this disorder, and in some centers, the sequence analysis of complete mitochondrial genome is used for definitive diagnosis of disease-related mutation in human mtDNA (18) which is also available in Iran, but we are still at the beginning of the way and we hope that later on diagnosis of patients would be made by combination of morphologic and genetic methods.



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