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Case Report

A Childhood Inflammatory Myopathy with Cytochrome Oxidase Deficiency: Which Came First, the Chicken or the Egg?

Sitokrom Oksidaz Eksikliği Olan Çocukluk Çağı Enflamatuvar Miyopatisi: Yumurta mı Tavuktan Çıkar, Yoksa Tavuk mu Yumurtadan?

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ABSTRACT

Inflammatory myopathies are autoimmune disorders rarely seen in childhood. Normally high-dose corticosteroid is the current treatment for inflammatory myopathies. For a specific subgroup of patients with inflammatory myopathy with cytochrome oxidase (COX)-negative myofibers that do not typically respond to corticosteroid treatment, and methotrexate (MTX) is used for therapy. Herein we present a 10-year-old girl who initially received clinical diagnosis of juvenile inflammatory myopathy which did not respond to corticosteroid treatment. Examination of her muscle biopsy specimen showed the presence of COX-negative muscle fibers which are very rarely seen in childhood inflammatory myopathies, and she was diagnosed as inflammatory myopathy characterized with COX-negative myofibers. The patient, who recovered with MTX therapy underwent genetic examination 3 years after the treatment was terminated. The sequence analyses of mitochondrial DNA (mtDNA) identified 19 variants in the rRNA, *ND2, ND4, ND5, COX1, COX3,* and *CytB* genes of the mtDNA of the patient and her mother. These mutations generally induce the production of synonym amino acids. However, four missense mutations on the *ND4, ATP6,* and *CytB* genes have caused structural changes in amino acids. None of these mutations have been previously reported as pathogenic variants. We have thought that these variations in such essential genes might destabilize mtDNA and could probably affect the ATP synthesis in our patient. Our final diagnosis was established based on abnormal inflammatory response induced by a hereditary mtDNA defect in a child with mitochondrial myopathy, rather than an inflammatory myopathy with COX deficiency.

Keywords: Childhood inflammatory myopathy, polymyositis with COX-negative myofibers, *ATP6* synthase gene, *ND4* gene, *CytB* gene, mitochondrial myopathy

ÖZ

Enflamatuvar miyopatiler, çocukluk çağında nadiren görülen otoimmün bozukluklardır. Normalde yüksek doz kortikosteroid, enflamatuvar miyopatiler için güncel tedavidir. Sitokrom oksidaz (COX) negatif miyofiberleri olan enflamatuvar miyopatili bir hasta alt grubu tipik olarak kortikosteroid tedavisine yanıt vermez. Bu durumda tedavi için metotreksat kullanılır. Burada başlangıçta klinik olarak juvenil enflamatuvar miyopati tanısı konulan ve kortikosteroid tedavisine yanıt vermeyen 10 yaşında bir kız çocuğu sunulmaktadır. Kas biyopsisinde çocukluk çağı enflamatuvar miyopatilerinde çok nadir görülen COX-negatif kas lifleri görüldü ve COX-negatif miyofiberli enflamatuvar miyopati tanısı aldı. Metotreksat tedavisi ile iyileşen hastada, tedavi kesildikten 3 yıl sonra genetik inceleme yapılabildi. Mitokondriyal DNA dizi analizlerinde hastanın ve annesinin mitokondrial DNA'sının rRNA, *ND2, ND4, ND5, COX1, COX3* ve *CytB* genlerinde 19 varyant tespit edildi. Bu mutasyonlar genellikle aynı amino asitlerin üretimine neden olmaktaydı. Ancak *ND4, ATP6* ve *CytB* genlerindeki dört missens mutasyon, amino asitlerin değişmesine neden olmuştu. Bu mutasyonların hiçbiri daha önce patojenik varyantlar olarak bildirilmemişti. Bu temel genlerdeki varyasyonların, mitokondrial DNA'nın kararsızlığına neden olabileceğini ve sunulan hastada ATP sentezini etkileyebileceğini düşündük. Genetik inceleme sonrası hastayı COX enzim defekti olan enflamatuvar bir miyopatiden çok, kalıtsal mitokondrial DNA kusurunun neden olduğu anormal enflamatuvar yanıt gözlenen mitokondrial miyopati olarak değerlendirdik.

Anahtar kelimeler: Çocukluk çağı enflamatuvar miyopatisi, COX-negatif miyofiberli polimiyozit, *ATP6* sentez geni, *ND4* geni, *CytB* geni, mitokondriyal miyopati

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INTRODUCTION

Inflammatory myopathies (IMs) with an autoimmune etiology are rarely seen in childhood[1,2]. Juvenile dermatomyositis, juvenile polymyositis and overlap myositis are the most frequent types of IMs. IMs are characterized with proximal muscle weakness, increased serum creatinine kinase (CK) levels, myopathic-patterns on electromyogram (EMG) and myopathy with inflammation detected during the histopathological examination of muscle biopsy specimens[2,3]. High-dose corticosteroids and methotrexate (MTX) are the current treatment modalities of IMs[2]. A subgroup of patients with IM with cytochrome oxidase (COX)-negative fibers typically do not respond to corticosteroid treatment. Furthermore, COX-negative patients can be misdiagnosed as a polymyositis due to lack of knowledge about this subgroup and lesser usage of COX- staining technique for biopsy specimens which delays the onset of preferred treatment with MTX[4,5].

COX is an enzyme encoded from mitochondrial DNA (mtDNA) in mitochondria that is responsible for electron transport chain. MtDNA deletions have been showed in 90% of patients with polymyositis which is characterized by the presence of COX-negative muscle fibers[4-6]. Contrary to the nuclear genome, the circular 16.6 kb (16,569 bp) mtDNA does not contain introns. The mtDNA has two strands. The heavy strand (H), which encodes for two rRNAs (12S rRNA and 16S rRNA), fourteen tRNAs, and twelve polypeptides, all of which are subunits of the respiratory chain complexes, and contains the majority of the information as follows: cytochrome b is composed of one complex III subunit, six complex I subunits (*ND1*, *ND2*, *ND3*, *ND4*, *ND4L*, and *ND5*), three complex IV subunits (*COI*, *COII*, and *COIII*), and two complex V subunits (ATPase 6 and ATPase 8). Nearly all of four subunits of complex II subunits are encoded by the nucleus[7-9].

Herein, we present a 10-year-old girl who was initially diagnosed as juvenile polymyositis which did not respond to corticosteroid treatment. Enzyme histochemical staining of muscle biopsy specimen demonstrated the presence of COX-negative muscle fibers that are very rarely detected in childhood. We also argue the dilemma: "Which came first: the chicken or the egg?"

CASE REPORT

In October 2010, a 10-year-old girl was presented to our outpatient clinic with complaints of pain in arms, shoulders, legs, knees, gait disorder and weakness in the arms and legs for 6 months which gradually worsened in the previous month. She was the second born baby of non-consanguineous parents, delivered at term with no significant perinatal problems. There is history of celiac disease and diabetes in her 14-year-old sister, and goiter in her mother. On physical examination, her body weight (59.5 kg: 97 p), height (149.5 cm: 90-97 p), body temperature (36 °C), pulse rate (90 bpm), respiratory rate (16/min), and blood pressure (125/80 mm/Hg: 50-90 p) were as indicated. She was conscious, oriented, and cooperated. Neurological examination revealed that bilateral proximal upper and lower extremity muscle strengths were 3/5, 4/5, respectively, bilateral deep tendon reflexes were symmetrically normal with flexor plantar responses. Rest of the physical examination findings were unremarkable. Laboratory test results were as follows: white blood cells: 6.600/mm^3, red blood cells: 4.000.000/mm^3, hemoglobin: 10.9 gr/dL, platelets: 213.000/mm^3, uric acid: 5.2 mg/dL (N: 3.8-5.8 mg/dL), aspartate aminotransferase: 240 U/L (10-40), alanine aminotransferase: 90 U/L (5-45), lactate dehydrogenase: 826 U/L (120-330), creatine kinase: 4694 U/L (5-130), creatine kinase MB: 106 U/L (0-20), C-reactive protein: 1.46 mg/dL (0.0-0.8), erythrocyte sedimentation rate: 32 mm/h (0-20), serum protein: 5.7 gr/dL (6.6-8.2), and serum albumin: 3.4 mg/dL (3.5-5.6). Patient was considered to have myopathy with increased serum muscle enzymes and proximal muscle weakness. Electromyographic findings were also compatible with a myopathic pattern. Magnetic resonance imaging revealed inflammatory process around proximal muscles of lower extremities. Patient was diagnosed as juvenile polymyositis, thereafter oral prednisolone (2 mg/kg/day) treatment was started. Muscle biopsy could not be performed priorly, due to the objection of her parents. Patient did not respond to steroid treatment. After second week of prednisolone treatment, pneumonia, urinary tract infection, multisystemic infectious disease and oral candidiasis were observed as complications of immune suppression. Since the patient did not respond to 18 days of prednisolone treatment, pulse steroid therapy was administered for 3 days, still without any clinical response. Thereafter, muscle biopsy was considered in consultation with her family and performed uneventfully. Histological examination of the biopsy specimen revealed only mild inflammatory infiltration that may be suppressed with corticosteroid treatment. In addition, inconspicuous areas of perifascicular atrophy (Figure 1) and numerous COX- negative muscle fibers visible with combined COX/succinate dehydrogenase (SDH) enzyme

histochemical staining were noted (Figure 2). Prednisolone dose was decreased, and MTX treatment (250 mcg/kg/week) was initiated. With MTX therapy her complaints decreased over several months and follow-up continued in outpatient clinics of pediatric immunology. She has been in good health for 7 years since the establishment of the diagnosis of COX-negative myopathy. The mtDNA sequence analyses revealed 19 variants in the rRNA, *ND2*, *ND4*, *ND5*, *COX1*, *COX3*, and *CytB* genes of the mtDNA of both patient and her mother. The variants of *m.8860A>G, Thr112Ala* and *m.9070T>G, Ser182Ala* variations at the *ATP6* gene, *m.10907T>G, Phe50Leu* at the *ND4* gene and *m.15326A>G, Thr194Ala* at the *CytB* gene were identified. None of these mutations have been reported as pathogenic variants previously[6-9]. Informed consent was obtained from the patient's parents.

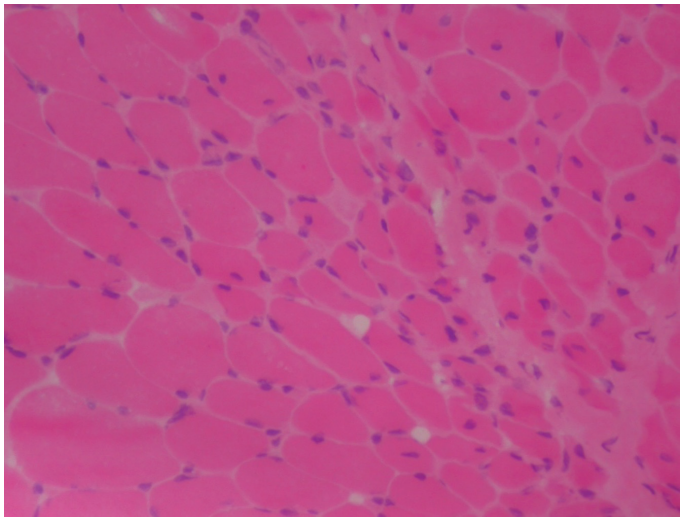

Figure 1. Presence of perifascicular atrophy which is often described in dermatomyositis (HEx100)

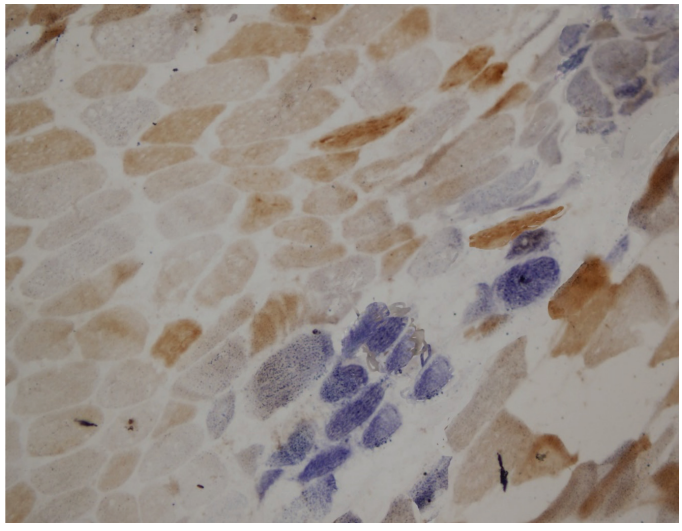

Figure 2. The "pathological blue myofibers" representing the presence of dysfunctional mitochondria (Combined COX/SDH stain x200)

COX: Cytochrome oxidase, SDH: Succinate dehydrogenase

DISCUSSION

The first report of polymyositis characterized with COX-deficient fibers, also known as PM with mitochondrial pathology (PM-Mito), was published in 1997[4]. It is a rare form of IM that shares clinical and pathological characteristics of sporadic inclusion body myopathy, such as a delayed age of onset, slow progression of quadriceps weakness, poor response to corticosteroids, endomysial inflammation with focal invasion of intact muscle fibers[4]. More precisely, the muscle biopsy should show diffuse HLA-1 upregulation, presence of >1% COX-negative fibers, lymphocytic endomysial infiltrates, particularly of CD8+ T-cells and/or macrophages invading non-necrotic fibers in this type of IM. But cytoplasmic inclusions and rimmed vacuoles ought to be absent[5]. IM with COX- negative fibers is usually recognized in adult patients who did not respond to long-term steroid therapy. Up to date, based on literature data, the patients with IM characterized by COX- negative muscle fibers were generally older than 50 years of age, and it has been rarely reported in children before[4-7]. All patients presented with proximal muscle weakness, abnormal EMG findings, and elevated serum CK values. Patients who typically had no clinical improvement with corticosteroid treatment, may be diagnosed as polymyositis with COX- negative muscle fibers based only on muscle biopsy findings[3,4]. Our patient was also presented with proximal muscle weakness, abnormal EMG findings, elevated serum CK values and did not show clinical response to steroid treatment which forced us to perform a muscle biopsy and then she was diagnosed as having inflammatory myositis with COX-negative muscle fibers. Clinical findings of our case were also compatible with the relevant literature data.

Examination of muscle biopsy specimens of these cases reveals inflammatory cell infiltrates, myopathic changes, complete absence of muscle fibers containing rimmed vacuoles and an excess of muscle fibers with deficient COX activity[3]. Myopathy characterized with muscle fibers of different sizes, focal invasion of muscle fibers by inflammatory cells, endomysial foci of CD4 and CD8 lymphocytes, excluding CD20 lymphocytes, positive cells and diffuse overexpression of MHC Class

I on the surfaces of muscle fibers identified throughout the muscle biopsy specimens are commonly seen during histopathological examination[5]. Inconspicuous inflammatory infiltration, excessive number of COX-negative muscle fibers and numerous "blue fibers" visualized with combined COX/SDH staining due to non-functioning mitochondria have been demonstrated during histopathological examination of the biopsy sample of our patient. There were no ragged red fibers, and rimmed vacuoles. Increased HLA class I expression was also observed.

Immunosuppressive therapy is the principal treatment in IMs. Corticosteroids are strongly recommended as the first-line immunosuppressive agents because of favorable treatment response achieved in IMs[1]. Unresponsiveness to steroid treatment has suggested that histochemical examination of muscle biopsy specimen might be useful to identify COX-negative IM patients as in our case. MTX is a treatment option for this group of patients, and this regimen is administered once weekly[3]. We initiated treatment with MTX (250 mcg/kg/week) for our patient and she responded over several months to this therapy. However, very often, the presence of COX-negative muscle fibers predicts a poor prognosis, even in cases treated with immunosuppressive treatment using MTX[3].

The nuclear genome is highly robust, while the typical mutation rate for the mitochondrial genome is 10-20 times greater. There are several explanations for this phenomenon, including the presence of unprotected histone proteins, a less effective mtDNA repair system relative to the nuclear repair system, and the close proximity of the respiratory chain to the mitochondria, which produces large amounts of reactive oxygen species, and exposes the mitochondrial genome to oxidative damage. This phenomenon has crucial importance during replication process because mtDNA is found in a single-stranded form for a prolonged period of time, making it more vulnerable to the assaults of radical oxygen species[5,6]. Numerous investigations have also demonstrated that mitochondrial mutations increase with ageing[6-9]. It has been also reported that mtDNA deletions occurred in 90% of the patients with IM characterized by COX-negative myofibers[4]. This finding aroused some suspicions indicating that mitochondrial dysfunction may induce pathologic inflammatory response, or an abnormal inflammatory response may be a contributory factor in mitochondrial dysfunction[6-9]. Due to the instability of mtDNA, the development of IMs characterized by COX-negative muscle fibers generally observed in elderly patients suggests possibly a coincidental association in some cases. The presence of multiple mutations in mtDNA in the presented case suggested that genetic examination is essential to elucidate the pathogenesis, especially in childhood IMs with COX-negative myofibers[6-9].

In conclusion, IM with COX-negative muscle fibers is a rare subtype of IMs in childhood[5]. COX-negative myopathy is a histopathologically proven myopathy. Pediatric patients with COX- negative muscle fibers are generally, and priorly receive corticosteroid therapy as the patients with polymyositis. However, in the presence of unresponsiveness to steroid therapy, muscle biopsy specimens should be obtained for the histopathologic diagnosis of COX-negative myopathy and genetic testing should be performed to disclose mtDNA mutations. Early initiation of MTX therapy should be kept in mind as a critical clinical decision to ensure a favorable treatment outcome in children with COX-negative myopathy. In the present case, genetic analyses have revealed different variants of several genes in the mtDNA. Same variants were also identified in her mother. These finding reminded us of the dilemma: "Which came first: the chicken or the egg?" Our final diagnosis was myositis due to an abnormal inflammatory response induced by hereditary mtDNA defects, rather than an IM with mitochondrial dysfunction.

Ethics

Informed Consent: Informed consent was obtained from the patient's parents.

Peer-review: Externally and internally peer reviewed.

Author Contributions

Surgical and Medical Practices: G.D., Ö.Y., Ü.B.Ş., Z.Y., C.A., C.Ö., A.B., Concept: G.D., Ö.Y., Ü.B.Ş., Z.Y., C.A., C.Ö., A.B., Design: G.D., Data Collection or Processing: G.D., Ö.Y., C.A., A.B., Analysis or Interpretation: G.D., A.B., Literature Search: G.D., Writing: G.D., Ö.Y., C.A., C.Ö.

Conflict of Interest: The authors have no conflict of interest to declare.

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