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A Case of Myasthenia Gravis Proven by Ultrastructural Study

Although light microscopic features of muscle are not pathognomonic in most cases of myasthenia gravis (MG), careful examination of neuromuscular junction by electron microscopy (EM) can reveal important clues for this disease. We report here a case of MG confirmed by EM study to emphasize that tissue diagnosis is still the best adjuvant to confirm the diagnosis. An 18-year-old female visited our hospital complaining of progressive muscle weakness for 3 years. She had difficulty in running, going upstairs and doing routine activities. Symptoms were aggravated with continuous work and resolved after rest. She had weakness of bilateral masseter and facial muscles and proximal portions of extremities without definite diurnal variation. Electromyography showed myopathic changes in proximal muscles of extremities. MG was considered but tensilon test was equivocal. Repetitive nerve stimulation tests revealed 20-30 percent decrease in responses to low and high rate stimulation. Muscle biopsy revealed selective type 2 atrophy. Ultrastructurally, abnormalities of neuromuscular junctions, i.e., wide primary synaptic cleft, and wide and shallow secondary synaptic clefts with mild myopathic features were present. These findings were pathognomonic for MG. Later, her symptoms were improved completely 3 months after thymectomy. The histologic finding of thymus was follicular hyperplasia.

Key Words: *Myasthenia Gravis; Microscopy, Electron; Biopsy; Muscle*

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INTRODUCTION

Myasthenia gravis (MG) is a well-defined autoimmune disease which goes against acetylcholine receptors (AChRs) in the neuromuscular junctions, resulting in impaired neuromuscular transmission. The main feature of MG is fluctuating weakness of voluntary muscles which is characterized by aggravated weakness during continuous activity, quick restoration of power after rest, and dramatic improvement in strength following the administration of acetylcholine esterase inhibitors (1). A decrease in the number of nicotinic AChRs and circulating antibody to the AChRs were present in nearly all patients with MG (2).

Despite the vast amount of MG findings by electron microscope (3-9), usually, the muscle biopsy is the last tool used to confirm MG. This tendency is derived not only from the invasiveness of muscle biopsy, but also the existence of other easy tests for making the diagnosis of MG, such as measuring antibody to the AChR, edrophonium (tensilon) test, or repetitive nerve stimulation test. In addition, light microscopic findings are often not remarkable and therefore, not diagnostic for MG. However, careful examination of the neuromuscular junction by EM provide us with robust features and should be used in the diagnosis of MG. We report here a case of MG, which showed atypical clinical features of MG, but characteristic typical ultrastructural findings.

CASE REPORT

A previously healthy 18-year-old female visited the Department of Neurology in our hospital complaining of progressive bulbar palsy and proximal weakness of extremities for 3 years. She had difficulty in running and going upstairs. She had also difficulty in doing routine activities such as combing hair, using cutlery, and brushing teeth for 3 years. She had several episodes of choking on liquids and collapsing during walking without loss of consciousness. Symptoms were aggravated with continuous work and fatigue, and resolved after a period of rest. There was weakness of bilateral facial and masseter muscles, and proximal portions of extremities on physical examination. Sensation of the patient was intact. Deep tendon reflexes were normal. Antinuclear antibody titer was 1:640 (homogeneous type), and anti-ds-DNA anti-

body level was 8.4 IU/mL (normal range: 0-7 IU/mL). CK and LDH were normal. Other laboratory tests including thyroid function tests were normal. Chest X-ray was unremarkable. Electromyographic study disclosed small amplitude polyphasic motor unit action potentials in right deltoid and vastus lateralis muscles during mild voluntary contraction, these findings were suggestive of myopathic changes. Repetitive nerve stimulation tests revealed definite decreased responses to low and high rate stimulation, posttetanic facilitation, and posttetanic exhaustion in the right abductor digiti minimi, flexor carpi ulnaris, and orbicularis oculi muscles. However, edrophonium (tensilon) test did not show definite improvement of weakness. A muscle biopsy was performed in the quadriceps femoris muscle.

RESULTS OF MUSCLE BIOPSY

Light microscopic findings

The muscle biopsy specimen showed selective type 2 fiber atrophy on myosine ATPase and NADH-TR stains (Fig. 1). Neither dystrophic changes nor inflammatory cell infiltration was present.

Electron microscopic findings

Two neuromuscular junctions were included in ultrathin section. Electron microscopic findings showed abnormalities of neuromuscular junctions with irregularly widened primary synaptic cleft up to 400 nm in maximum width. The secondary synaptic clefts were also irregularly widened and shallow, and partly reduced in number. Schwann cells partially wrapped the nerve terminal (Fig. 2). The motor nerve ending contained about 50 synaptic vesicles per μm^2 which were normal in number. Mitochondria in nerve terminal were enlarged with rarefaction of the cristae (Fig. 2, 3). Focal rarefaction of myofilaments and focal accumulation of mitochondria (Fig. 4) were also observed in the subsarcolemmal areas within the secondary synaptic clefts. Reduplicated basal lamina materials were also observed (Fig. 4, 5).

Follow-up

After tissue diagnosis of MG, serum level of antibody to AChR was checked and it had a very high titer of 16.1 nmol/L (normal range: <0.15 nmol/L). The patient improved in chewing and walking after oral pyridostigmine and prednisolone therapy. Computed tomography (CT) scan of the thorax revealed subinvoluted thymic tissue. Thymectomy was performed. The thymus was yellow and soft and $9\times7\times1$ cm in size and 25 g in weight. It showed thymic follicular hyperplasia with thymic cyst. Most of her muscular symptoms were dramatically improved 3 months after thymectomy.

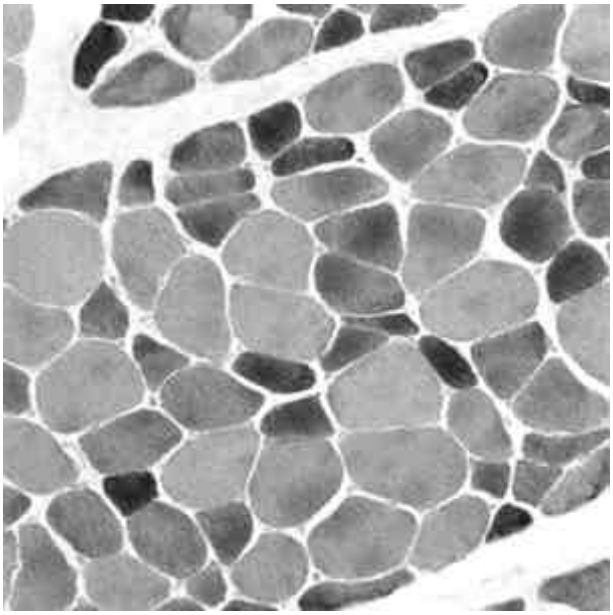

Fig. 1. Myosin ATPase at pH 9.4 stain shows selective type 2 atrophy. Dark brown fibers are atrophic type 2 fibers and pale fibers are type 1 (×100).

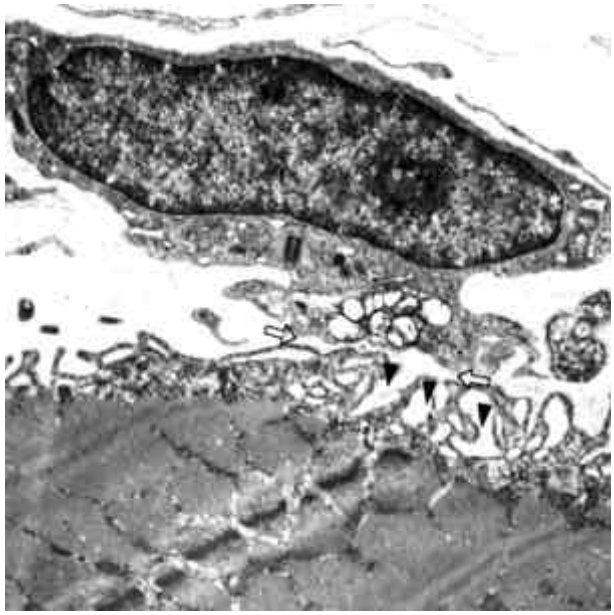

Fig. 2. A neuromuscular junction shows irregularly widened primary (arrow) and secondary synaptic clefts (arrowheads). Secondary synaptic clefts are irregularly shallow, and partly reduced in number. S, Schwann cell; A, axon terminal (Uranyl acetate and lead citrate stain, ×5,000).

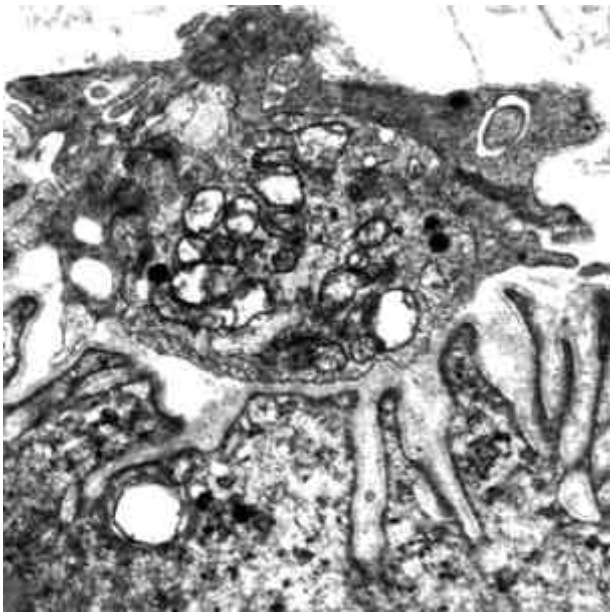

Fig. 3. Mitochondria in nerve terminal are enlarged with rarefaction of cristae (Uranyl acetate and lead citrate stain, × 6,000).

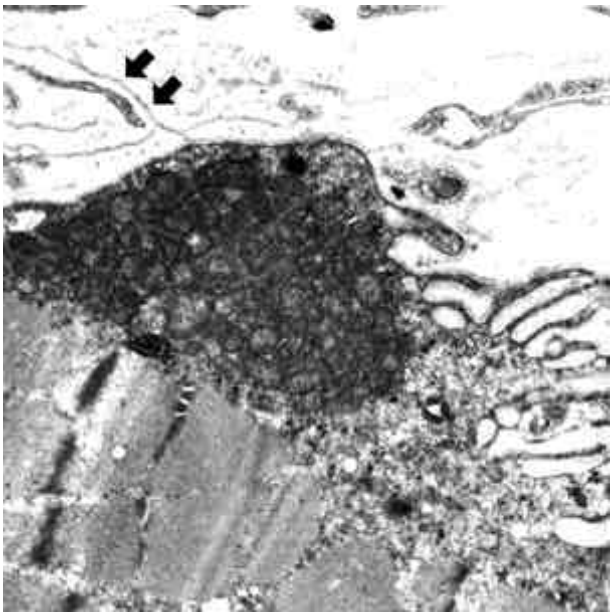

Fig. 4. Mitochondrial accumulation in the subsarcolemmal area is seen. Reduplicated external lamina (arrows) are present (Uranyl acetate and lead citrate stain, ×5,000).

DISCUSSION

The pathogenesis and pathologic findings of MG are well elucidated. Light microscopic examination of muscle biopsy of our case revealed selective type 2 fiber atrophy. Although type 2 fiber atrophy is a nonspecific finding that can be observed in malnutrition, cachexia, steroid

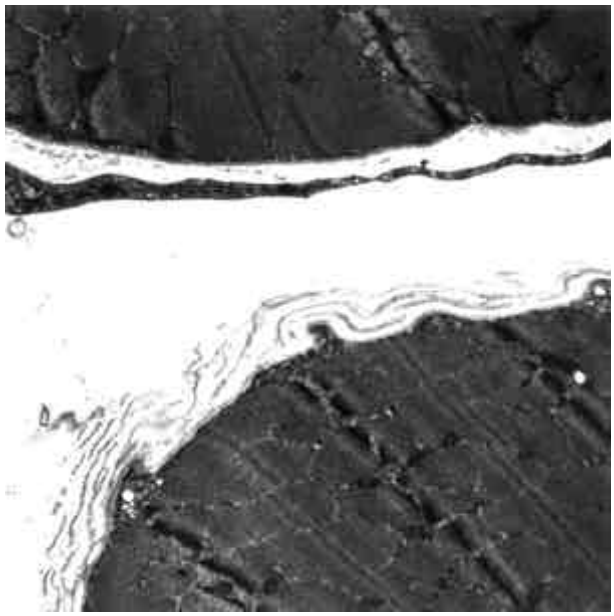

Fig. 5. Multiple layers of reduplicated basal lamina materials are remarkable in atrophic myofibers (Uranyl acetate and lead citrate stain, ×3,000).

induced myopathy, disuse atrophy, Parkinson's disease, and collagen vascular disease (10), it can only be features of MG under light microscopy. Engel and McFarlin found type 2 atrophy in about half of their patients with MG (8) and they speculated this finding as denervation atrophy. Denervation is usually associated with myasthenic damage to motor end-plates, but it has been shown that treatment with anticholinergic drug itself causes morphological and functional changes in motor end-plates (11). However, the reason why type 2 specific atrophy develops is still unknown.

The normal neuromuscular junction is known to consist of 5 principal components, including Schwann cell process, nerve terminal, synaptic space, postsynaptic membrane and junctional sarcoplasm. The normal nerve terminal contains mitochondria, neurofilaments, microtubules, a few smooth endoplasmic reticulum and abundant synaptic vesicles. Glycogen granules, lysosomes, and larger canaliculi, and cisternae can be present. The synaptic space is situated between presynaptic and postsynaptic membranes. The primary synaptic cleft is limited by the presynaptic membrane on one side and by an imaginary plane tangential to the terminal expansions of the junctional folds on the muscle side. For efficient neuromuscular transmission, the primary synaptic cleft is narrow, approximately 70 nm wide normally. The secondary synaptic clefts are spaces between the junctional folds, and each secondary synaptic cleft communicates with the primary synaptic cleft. They also have regular width and length in normal state. The mean ratio of presynaptic

to postsynaptic membrane areas ranges from 1:8 to 1:10 (10).

Electron microscopic abnormalities of MG have been discovered from the end of 1960 after the introduction of EM (3-7). Widening of primary and secondary synaptic clefts was first described in on MG patient in 1962 (6). Reduced number of junctional folds, increased synaptic vesicles, and dense mitochondria at expanded axon terminal were described by Woolf (3). Thereafter, similar observations have appeared in the papers (7). Engel and Santa (4) reported a significant decrease in mean terminal area and the mean postsynaptic membrane profile, and widened, sparse and shallow or sometimes absent secondary synaptic clefts. Bergman et al. (5) described considerable variation in the number of synaptic vesicles and rarefaction of the cristae of mitochondria in nerve terminal, partial wrapping of the junctional site by Schwann cells, and subsarcolemmal aggregation of mitochondria and T-tubules in addition to shallow and wide secondary clefts. In our case, about 50 synaptic vesicles per μm^2 of the nerve terminal area were observed, which considered normal in number (normal range; 50 to 70 synaptic vesicles/μm^2 of the nerve terminal area) (10).

The changes in myofibers were also described in MG such as increased intermyofibrillar space, reduction in myofibril size, myofibrillar disorganization and the accumulation of nuclei and mitochondria adjacent to the capillaries with swollen endothelial cells and thickened capillary basement membrane (5). Korenyi-Both et al. (9) described increased amount of basement membrane material.

Ultrastructurally, most of the previously described findings were observed in our case, but there were neither aggregation of T-tubules nor swollen endothelial cells and thickened basement membranes. In atrophic myofibers, multiple layers of reduplicated external lamina were prominent, signifying repeated episodes of atrophy and regeneration.

Although characteristic ocular findings, such as ptosis and diplopia were not prominent in our case, difficulty in swallowing and progressive proximal muscular weakness of extremities were present, which were aggravated by continuous work and resolved after a period of rest. From these clinical manifestations, MG was considered, despite an equivocal edrophonium (tensilon) test. Therefore, to rule out other combined neurogenic atrophy or primary myopathic diseases, a muscle biopsy was performed.

Usually, light microscopic features of muscle specimen from the patients with MG are not remarkable and even in some biopsies, little or no abnormality is present. Even though an EM study of muscle biopsies was performed, if it did not include neuromuscular junctions in the sampling for EM, the confirmative diagnosis would have been difficult. We fortunately found neuromuscular junctions on semithin section, and the diagnosis of MG was possible. Therefore, looking at the neuromuscular junction is important. This case suggests that the tissue diagnosis of EM is still the best adjuvant tool to confirm MG in mild cases or in patients with atypical clinical manifestation of MG.

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