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【 CASE REPORT 】

Recurrent HyperCKemia with Immunological Involvement of the Endomysial Capillaries in Neuromyelitis Optica

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Abstract:

A 55-year-old woman with neuromyelitis optica (NMO) had recurrent myalgias with hyperCKemia. A muscle biopsy suggested nonspecific myopathic changes. Regarding immunohistochemistry, the expression of both major histocompatibility complex class I and myxovirus resistance protein A was observed in the endomysial capillaries, suggesting immunological involvement of these capillaries, whereas both C5b9 (membrane attack complex) and aquaporin 4 immunofluorescence stainings were normal. The present findings led us to conclude that one possible mechanism for hyperCKemia in NMO underlying the immunological involvement of the endomysial capillaries was an as-yet-unidentified factor that triggered damage to the integrity of the sarcolemma and thereby cause CK leakage into the serum.

Key words: endomysial capillaries, hyperCKemia, major histocompatibility complex class I, myxovirus resistance protein A, neuromyelitis optica

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Introduction

Neuromyelitis optica (NMO) is an autoimmune demyelinating disease of the central nervous system (CNS) characterized by severe optic neuritis and longitudinal myelitis (1). An antibody for aquaporin 4 (AQP4) is detected in the serum of patients with NMO. AQP4-IgG is known to cause damage to both the CNS and muscle (2). Indeed, an association has been reported between elevated creatine kinase activity in the serum (hyperCKemia) and NMO; however, the pathological findings in that study were limited (2).

We herein report a rare case of a patient with NMO showing recurrent hyperCKemia with immunological involvement of the endomysial capillaries.

Case Report

A 55-year-old Japanese woman with no remarkable medical history presented with vomiting plus pain in her left leg. Laboratory tests revealed hyperCKemia (2,240 U/L). Within 14 days after her presentation, her clinical symptoms spontaneously remitted, and her CK level became normal (36 U/L). At 1 month later, she showed vomiting and pain in both arms and both legs, followed by bulbar palsy. The CK level was normal (39 U/L). A cerebrospinal fluid (CSF) analysis revealed mild lymphocytic pleocytosis (7 cells/mm^3) and no oligoclonal bands. Magnetic resonance imaging (MRI) revealed lesions in the left temporal horn, left pontine tegmentum, and left medulla oblongata but no spinal lesions (Fig. 1A). The results of an anti-AQP-4 antibody test by an enzyme immunoassay were negative (2.0 U/mL; cut-off 3.0 U/mL). The results of the following tests were all negative: anti-nuclear antibody, anti-ds-DNA, SS-A, SS-B, and anti-myelin oligodendrocyte glycoprotein-antibody. We made a diagnosis of a CNS demyelinating disease with a likely autoimmune etiology. We started a 3-day course of intravenous methylprednisolone (1 g/day), and the patient's symptoms then gradually improved.

However, three months later, the patient again experienced diffuse pain in her arms and legs. The muscle pain began gradually with no apparent trigger points, indicating myal-

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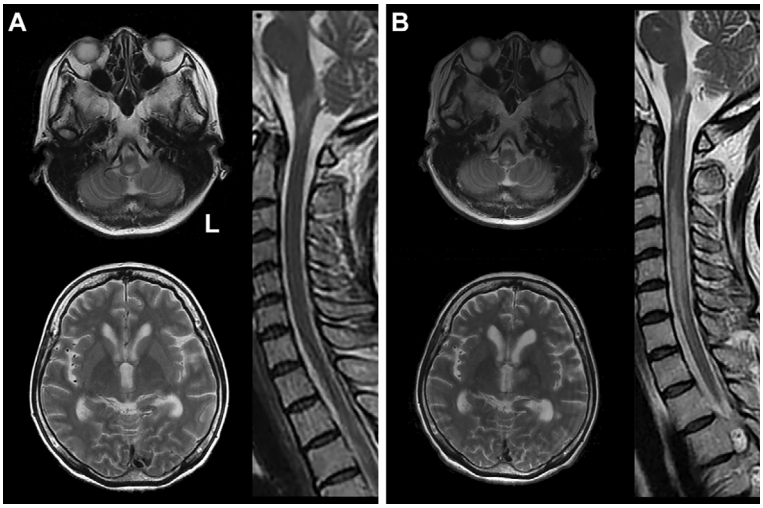

Figure 1. **Brain MRI findings. A: Initial MRI (T2-weighted images) performed when the patient showed no hyperCKemia. Hyperintense areas were observed in the left temporal horn, left pontine tegmentum, and left medulla oblongata, whereas no abnormalities were observed in the spinal cord. B: The second MRI procedure performed when the patient showed hyperCKemia. Hyperintense areas were observed in the spinal cord extending from C2 to T1 as well as in the left thalamus and left medulla oblongata.**

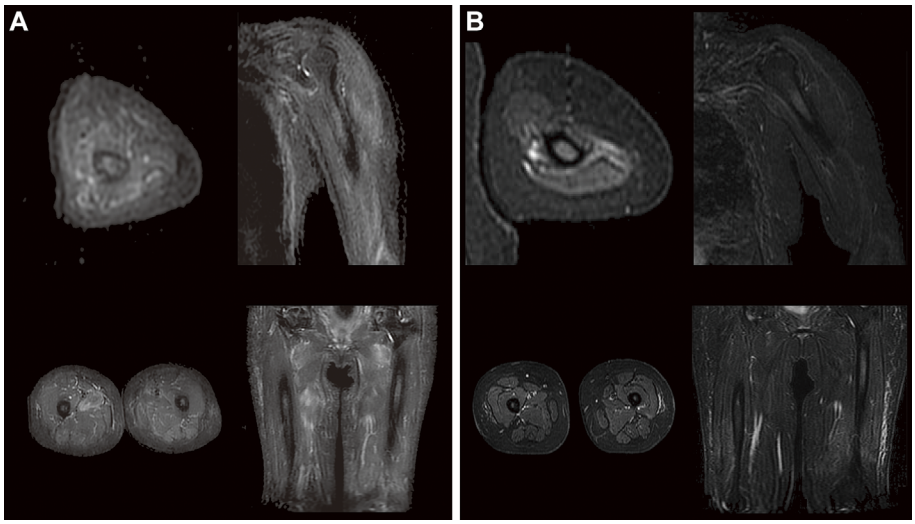

Figure 2. **Muscle MRI findings. A: Muscle MRI (STIR images) performed when the patient showed hyperCKemia. Hyperintense areas were observed in the left triceps, biceps, and femur. B: The second MRI procedure performed after the improvement of hyperCKemia. The abnormal hyperintensity in the initial MRI was resolved. STIR: short tau inversion recovery**

gia. Her CK level was elevated (15,246 U/L). Muscle MRI showed hyperintensities in the left triceps, biceps, and femur, suggesting myopathy (Fig. 2). An electromyogram showed myogenic changes. We then performed a muscle biopsy of the patient's left biceps (Fig. 3, 4).

Stored frozen muscle samples were processed into 10-μm sections for routine histochemistry and immunohistochemistry. For immunohistochemistry, sections were washed with phosphate-buffered saline (PBS) and incubated with primary antibody for 1 hour in a humidified chamber at room temperature. After washing with PBS, the slides were incubated for 30 minutes with diluted biotinylated secondary antibody. After the secondary incubation, the slides were incubated for 30 minutes with an avidin/biotin-based peroxidase system (Vectastain Elite ABC-kit Standard, Vector Laboratories, Burlingame, USA) and visualized by incubating with a peroxidase substrate solution. For primary antigens, antibodies against major histocompatibility complex (MHC) class I (1:100 dilution; Dako, Glostrup, Denmark), myxovirus resistance protein A (MxA; 1:200 dilution, Abcam, Cambridge,

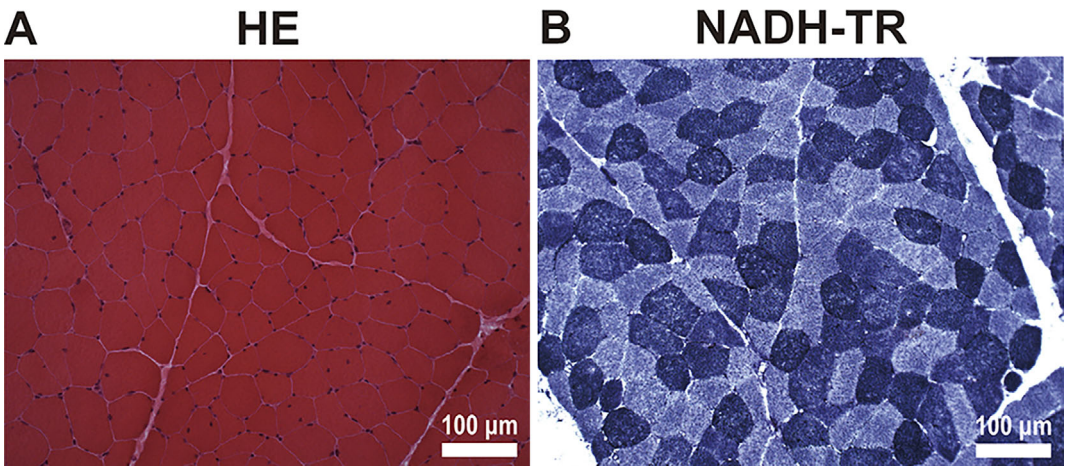

Figure 3. **Histological findings of a muscle biopsy. A: Hematoxylin and Eosin staining showed minimal variation in fiber size, no degenerating or regenerating muscle, and no endomysial fibrosis. Endomysial and perivascular inflammatory infiltrates were also not seen. B: NADH-TR staining showed intermyofibrillar network disorganization.**

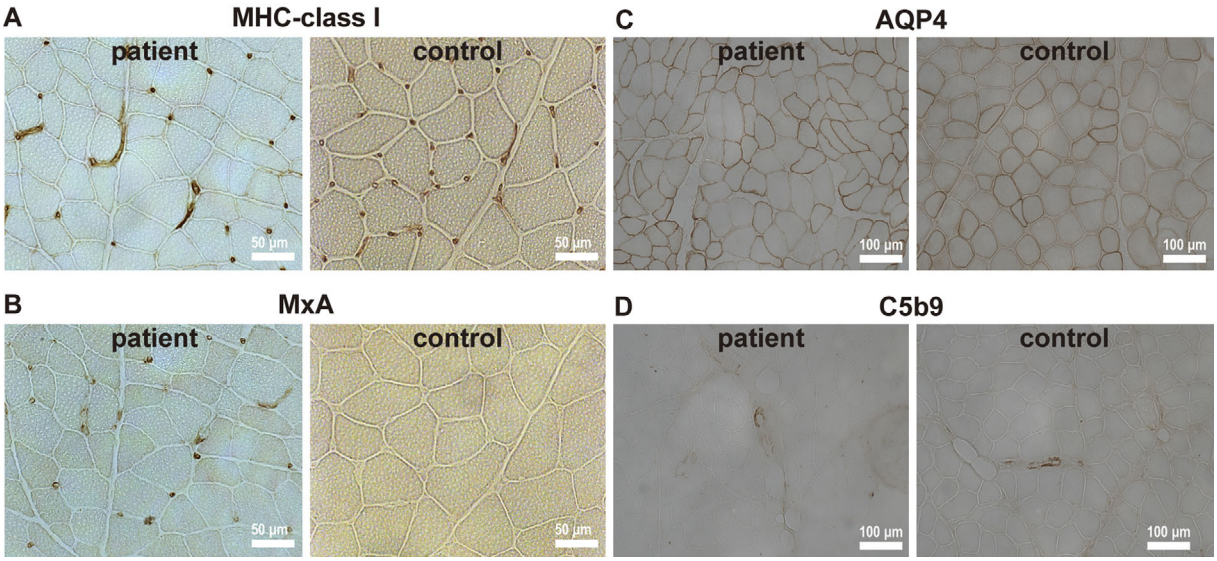

Figure 4. **Immunohistochemistry findings of a muscle biopsy. A, B: Endomysial capillaries were stained by both MHC-class I and MxA staining, suggesting their immunological involvement in endomysial capillaries. C, D: AQP4 and C5b9 immunofluorescence stainings were normal.**

UK), AQP4 (1:1500 dilution, AB3594; Chemicon International, Temecula, USA), and C5b-9 (1:100 dilution; Dako) were used. Hematoxylin and Eosin (HE) staining showed minimal variation in fiber size, no degenerating or regenerating muscle, and no endomysial fibrosis (Fig. 3A). Endomysial and perivascular inflammatory infiltrates were also not seen. NADH-tetrazolium reductase (NADH-TR) staining showed intermyofibrillar network disorganization (Fig. 3B). These findings suggested nonspecific myopathic changes.

Immunohistochemistry revealed the expression of both MHC class I (Fig. 4A) and MxA (Fig. 4B) in endomysial capillaries, suggesting immunological involvement of the endomysial capillaries. The results of staining with AQP4 immunofluorescence (Fig. 4C) and with C5b9 (membrane attack complex) (Fig. 4D) were normal.

The patient suffered a loss of consciousness and severe paraparesis. A CSF analysis revealed lymphocytic pleocytosis (56 cells/mm^3) and no oligoclonal bands. MRI revealed new lesions in the left thalamus and left medulla oblongata, as well as a spinal cord lesion extending from C2 to T1 (Fig. 1B). The result of another anti-AQP4 antibody test was positive (≥40 U/mL). We made a diagnosis of NMO and initiated treatment with intravenous methylprednisolone (1 g/day) for 5 days and seven courses of plasma exchange, followed by oral prednisolone (40 mg/day) and azathioprine immunotherapy.

All of the patient's symptoms gradually improved. Her CK level became normal (55 U/L), and her anti-AQP-4 antibody result became negative (≤1.0 U/mL). The abnormal findings of her MRI were resolved (Fig. 2B), and an electromyogram showed no abnormalities.

Discussion

We reported the pathological findings in a rare case of recurrent hyperCKemia in NMO that demonstrated immunological involvement of the endomysial capillaries. Of note, the expression of MxA and MHC-class I was observed in the endomysial capillaries in this patient, whereas the AQP4 immunofluorescence staining was normal.

The results of a muscle biopsy for hyperCKemia in NMO have been reported in four cases (3-5). In each of these cases, HE staining showed normal findings or nonspecific changes with no necrosis, which is compatible with the histological findings in our present case (Fig. 3A). Regarding the AQP4 immunoreactivity, in three of the cases immunoreactivity on the muscle plasma membrane was lost with local depositions of sarcolemmal IgG and activated complement membrane attack complex (4, 5). Based on these findings, it was speculated that the mechanism underlying hyperCKemia is related to CK leakage caused by damage to the integrity of the sarcolemma rather than by muscle necrosis (2). However, the AQP4 immunofluorescence staining was normal in our patient's case (Fig. 4C) as well as in another previous case (3). We therefore presumed that the muscle biopsies in the previous case and in our patient had been performed before the damage to the integrity of the sarcolemma had occurred.

The expression of MHC-class I and MxA was observed in our patient's endomysial capillaries (Fig. 4A, B). MHC-class I overexpression is often observed in immune inflammation myopathy, and normal muscle fibers do not express this antigen (6). MxA is a type 1 interferon-inducible protein that normally functions in the defense against viral infections through a variety of means, such as the inhibition of viral transcription and translation and assembly of viral nucleocapsids. MxA has been consistently observed in the myofibers of dermatomyositis patients in regions of perifascicular atrophy and in the endothelium of endomysial capillaries (7). The expression of capillary MxA protein has been considered to occur early in the course of muscle weakness and was present in dermatomyositis patients who had not received treatment at the time of their biopsy (7). Taken together, the past and present findings led us to conclude that one possible mechanism for hyperCKemia in NMO underlying the immunological involvement of the endomysial capillaries was an as-yet-unidentified factor that triggered damage to the integrity of the sarcolemma, resulting in CK leakage into the serum.

The major CK isoform of muscle is located in the cytoplasm and is largely not bound to the cytoskeleton (8). AQP 4 is anchored in the sarcolemma as a component of the dystrophin-associated protein complex linking the cytoskeleton to the extracellular matrix. Indeed, sarcolemmal damage and hyperCKemia are characteristic of dysferlinopathy, a condition in which membranous AQP4 is reduced (9). Mild endomysial inflammation in the skeletal muscle was observed in a case with the loss of AQP4 immunoreactivity (4). We speculated that the following mechanism might underlie the involvement of hyperCKemia in NMO: endomysial inflammation is triggered by the immunological involvement of the endomysial capillaries, which in turn leads to the loss of AQP4 in the sarcolemma, and then structural disorganization in the sarcolemmal membrane due to the loss of AQP4 results in CK leakage into serum. Further immunohistochemical studies will be needed to clarify the mechanism underlying the role of hyperCKemia in NMO.

In conclusion, we presented the pathological findings of a case of recurrent hyperCKemia in NMO that showed immunological involvement of the endomysial capillaries. Most cases of hyperCKemia in NMO show recurrent CK elevation (3-5, 10-12), and hyperCKemia can precede CNS demyelinating attacks. Indeed, our case also showed repeated hyperCKemia before CNS demyelinating attacks. The relapsing form of NMO leads to severe, permanent, relapse-related neurologic impairment within five years, so an early diagnosis is crucial (1). Although hyperCKemia is a rare condition for patients with NMO, it is a possible indicator of CNS demyelinating attacks in NMO.

The authors state that they have no Conflict of Interest (COI).

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