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TRANSIENT HYPERCKEMIA IN THE SETTING OF NEUROMYELITIS OPTICA (NMO)

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Abstract

Introduction—Neuromyelitis optica (NMO) is characterized by inflammatory demyelinating lesions of the spinal cord and optic nerves from an autoimmune response against water channel aquaporin-4 (AQP4). We report 2 patients with transient hyperCKemia associated with NMO suggesting possible skeletal muscle damage.

Methods—Patient 1 was a 72-year-old man who presented with muscle soreness and elevated serum creatine kinase (CK) preceding an initial attack of NMO. Patient 2 was a 25-year-old woman with an established diagnosis of NMO who presented with diffuse myalgias, proximal upper extremity weakness, and hyperCKemia. Muscle biopsies were obtained for histopathologic evaluation, protein gel electrophoresis, immunofluorescence, and complement staining.

Results—In both patients the muscle showed only mild variation in fiber diameter. There were no inflammatory changes or muscle fiber necrosis, though there was reduced *AQP4* expression and deposition of activated complement.

Conclusions—Complement-mediated sarcolemmal injury may lead to hyperCKemia in NMO.

Keywords

aquaporin 4; creatine kinase; hyperCKemia; immunofluorescence; neuromyelitis optica

Neuromyelitis optica (NMO) is an inflammatory demyelinating disease of the central nervous system (CNS) with selective involvement of the spinal cord and optic nerves. Circulating immunoglobulin (IgG) antibodies against the astrocyte water channel protein,

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aquaporin-4 (*AQP4*), are present in the majority of NMO patients and are thought to be pathogenic once they enter the CNS.[1] A complement-mediated mechanism of astrocyte cytotoxicity is thought to play a role in the pathogenic event that is characterized by inflammation, blood–brain barrier disruption, oligodendroglial injury, and demyelination.

In addition to the CNS, *AQP4* is expressed in the kidney collecting duct epithelium, gastric parietal cells, airway epithelium, exocrine glands, and fast twitch skeletal muscle fibers.[2] Although there is a notable absence of pathology in NMO in peripheral organs, there have been a few reports of skeletal muscle damage associated with NMO.[3,4] Here, we report 2 patients with NMO associated with hyperCKemia with analyses of *AQP4* expression in muscle biopsies.

CASE REPORTS

Patient 1

A 72-year-old man with hyperlipidemia and atrial fibrillation presented to the emergency department with a 1-month history of easy fatigability. He reported 3 days of mild myalgias, tachycardia, and dyspnea on exertion. Chest X-ray revealed bilateral infiltrates. He was subsequently admitted for presumed community acquired pneumonia. The muscle soreness prompted an analysis of serum total creatine kinase (CK) that was found to be elevated at 66,000 U/L. Simvastatin, which he had taken for at least 4 years, had recently been increased in dosage from 10 mg to 20 mg daily. This medication was discontinued, and he received aggressive fluid and bicarbonate replacement.

Over the next 4 days he developed mild proximal leg weakness that progressed to involve his arms. CK levels rose to 147,000 U/L. Alanine and aspartate liver transaminases were elevated to 599 U/L and 1870 U/L, respectively. CK-MB was normal. A livedo reticularis like rash erupted over his knees and thighs. Magnetic resonance imaging (MRI) of the thighs showed changes consistent with diffuse intramuscular edema. Glucocorticoids were initiated, and a left quadriceps muscle biopsy was obtained the next day. Over the next week, the CK dropped to 234 U/L. Normal or negative studies included TSH, antinuclear antibody, antineutrophil cytoplasmic antibody, anti-Jo1, anti-SSA, anti-SSB, anticardiolipin, serum protein electrophoresis, cryoglobulin, Venereal Disease Research Laboratory serology, and antibodies against human immunodeficiency virus, hepatitis C virus, *Borrelia* burgdorferi, West Nile virus, human T-cell lymphotrophic virus-1, and *Mycoplasma*.

On hospital day 7, the patient required endotracheal intubation for respiratory distress and was transferred to the intensive care unit for monitoring. Within the next 48 hours, he developed progressive ascending paresis and numbness. He had mild facial paresis, torsional nystagmus, and flaccid quadriplegia with only trace movements of the deltoids. In addition, he was areflexic and had a sensory level up to T4. CSF analysis revealed 3 white blood cells and a protein level of 103 mg/dl. MRI of the spine showed diffuse high T2 signal extending from C2 to T11 with no significant enhancement. MRI of the brain showed nonspecific periventricular white matter changes with a few areas of restricted diffusion in the cortex, pericallosal region, cerebellum, and medulla, which were likely embolic infarcts of cardiac origin. An electromyogram (EMG) was performed on the thirteenth day of admission that

showed fibrillation potentials in multiple muscles of the left upper and lower extremities but no voluntary motor unit action potentials.

Subsequently, an anti-AQP4 antibody assay was found to be positive in both serum and CSF, and a diagnosis of NMO was made. Plasmapheresis and a trial of rituximab failed to produce clinical improvement. The patient underwent tracheostomy and had a prolonged hospitalization with multiple infectious complications. Eventually a decision was made to de-escalate care, and the patient died.

Patient 2

A 22-year-old woman with *AQP4* antibody positive NMO who was clinically stable while receiving rituximab therapy between November 2008 and August 2009 presented in April 2010 with complaints of myalgias. On exam there was mild proximal weakness of the upper extremities. An elevation of liver transaminases was noted, and a serum CK value >20,000 U/L was obtained. There was no myoglobinuria. Other workup included negative antinuclear antibody, anti-SSA, anti-SSB, rheumatoid factor, anti-cardiolipin antibodies and antibodies against human immunodeficiency virus, Hepatitis B, and Hepatitis C. TSH was not obtained at this time. EMG showed low-density fibrillation potentials in the left tibialis anterior muscle and short duration, low amplitude motor unit action potentials in the left deltoid and triceps muscles. Early recruitment pattern was noted in left deltoid, biceps, and triceps muscles. A right biceps muscle biopsy was performed. She received 2 infusions of rituximab in June 2010 with normalization of the serum CK and resolution of muscle aches.

In November 2011, she presented to the emergency department with 1 week of paresthesias and weakness of the left arm and right leg. On exam she was noted to have mild distal weakness of the left upper extremity with impaired wrist extension, finger extension, and finger abduction and flexion (Medical Research Council [MRC] grade 41). In addition, she had impaired right knee flexion and extension and ankle dorsiflexion and plantar flexion (MRC grade 4). There were no pathological reflexes. CK was once again found to be elevated to 1918 U/L. Cervical imaging revealed an extensive cord signal abnormality from C3–T2 with focal enhancement at C5 (Fig. 1). She was given 2 days of intravenous steroids and discharged home on a prednisone taper. Rituximab was reinitiated, and a CK level obtained 3 months after discharge was within normal limits.

MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded sections of muscle biopsies were stained with hematoxylin and eosin stains (H&E). H&E, trichrome, ATPase, nicotinamide adenine dinucleotide (NADH), and succinate dehydrogenase stains were performed on frozen sections. Adult skeletal muscles from 2 patients without a history of neoplasia, autoimmune disease, or systemic infection were used as controls.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and blue native/PAGE (BN-PAGE) were done on homogenates from frozen skeletal muscle. For BN-PAGE, homogenates were suspended in blue native lysis buffer, and 10 micrograms of protein sample were mixed with 5% Coomassie blue G-250 and loaded in each lane. Laemmli SDS-

PAGE gels consisted of a 12% running gel and 3% stacking gel. Following electrophoresis, as described previously,[5] proteins were blotted onto polyvinylidenedifluoride membranes (Millipore) for *AQP4* immunoblot.

For immunofluorescence, paraffin sections were de-waxed twice in xylene and rehydrated using graded ethanols. After immersion in blocking buffer (1% bovine serum albumin [BSA]), sections were immunostained at room temperature for 1 hour with rabbit anti-*AQP4* (1:200, Santa Cruz Biotechnology, Santa Cruz, CA) followed by fluorescent goat anti-rabbit and anti-human IgG secondary antibodies (1:200, Invitrogen). Tissue sections were examined with a Leica (Wetzlar, Germany) DM 4000 B microscope.

Complement staining was performed on frozen sections that were thawed and incubated in PBS for 10 min, blocked in 1% BSA for 1 h, followed by incubation with mouse anti-C5b-9 antibody (clone aE11, dilution 1:100 in blocking buffer; Santa Cruz Biotechnology) for 1 h. Sections were then washed and incubated with a fluorescent goat anti-mouse secondary antibody (1:200 in blocking buffer, Invitrogen). Tissue sections were examined with a Leica (Wetzlar, Germany) DM 4000 B microscope. Skeletal muscles from 3 known cases of dermatomyositis were used as positive controls, and normal skeletal muscle was used as negative control.

RESULTS

Muscle biopsies from both patients demonstrated similar histopathological findings. H&E sections (Fig. 2) showed minimal variation in fiber size, no degenerating or regenerating muscle fibers, and no endomysial fibrosis. Endomysial and perivascular inflammatory infiltrates were also not seen. NADH staining showed no moth-eaten fibers, no scalloping of the myofibrillar matrix, and no increase in subsarcolemmal crescents.

Immunofluorescence revealed decreased *AQP4* immunoreactivity on the muscle plasma membrane in both patients (Fig. 3A). Increased IgG binding was demonstrated in patient 2 (Fig. 3B). *AQP4* immunoblot analysis following SDS-PAGE of muscle homogenates showed reduced *AQP4* protein expression (Fig. 4A). *AQP4* immunoblot following BN-PAGE, which is able to reveal *AQP4* tetramers and orthogonal arrays of particles (OAPs) of different sizes, showed relatively more *AQP4* supramolecular aggregates in the NMO patient biopsies (Fig. 4B).

Staining for activated complement using an antibody that recognizes C5b-9 (membrane attack complex) was positive in both patients, with stronger sarcolemmal staining in patient 2 than in patient 1 (Fig. 3C).

DISCUSSION

We report 2 patients with transient, marked hyperCKemia associated with NMO. In both patients, the histopathological findings on light microscopy were remarkably minimal, given the very high CK values. While it is possible that sampling errors explain the minimal findings, the reduced amounts of AQP4/OAPs in the biopsy specimens suggest an

association between NMO and hyperCKemia. We suspect an immunemediated cause for the CK elevations.

AQP4 monomers consist of 8 helical membrane-associated segments surrounding an aqueous pore.[1] The monomers aggregate into tetramers and further into OAPs. In NMO, *AQP4*-IgG binds preferentially to OAPs, which are crucial for complement-mediated cytotoxicity.

AQP4 is expressed widely in peripheral organs that do not have the equivalent of the blood–brain barrier. In mice, *AQP4*-IgG was found to bind rapidly to *AQP4* in peripheral organs expressing *AQP4*.[6] It is unclear why substantial peripheral organ damage is absent in NMO. Recently, however, some evidence suggests possible muscle disease associated with NMO. In a retrospective study by Suzuki and colleagues, 3 patients with generalized fatigue were noted to have significant, transient, hyperCKemia preceding the onset of optic neuritis.[3] Subsequently, other reports documented an elevation in CK preceding NMO attacks as well as in isolation.[4,7,8] *AQP4* expression has been shown to be greatly reduced in Duchenne and Becker muscular dystrophies, sarcoglycanopathies, and dysferlinopathies.[9,10] However, a role for diminished expression of *AQP4* in the pathogenesis of these degenerative muscle diseases is not clear.

Muscle tissue from 2 other reported NMO-hyperCKemia cases, like ours, had no significant pathological findings on light microscopy.[7,8] It seems likely that minimal histopathology is a feature of NMO-associated hyperCKemia. The hyperCKemia in these patients may not come from muscle necrosis. One hypothesis to explain these results is that complement-mediated sarcolemmal injury leads to CK leakage without subsequent muscle fiber necrosis. This is supported by sarcolemmal staining for activated complement (membrane attack complex) in our 2 patients. Studies have shown *AQP4*-IgG binding to AQP4 causes complement-dependent cytotoxicity in human cell lines and mouse models.[11,12] The relative lack of inflammatory cells indicate a different mechanism of damage in muscle compared with the processes leading to CNS injury. In the CNS, microglia play an important role in the neuroinflammatory cascade. The limited extracellular space in the CNS versus muscle may lead to greater confinement of inflammatory cells and cytokines, which in turn leads to more injury.[1]

It is unclear whether the reduced amount of *AQP4* tetramers/OAPs precedes the immune attack or is a consequence of it, though the latter explanation seems more likely. Rapid internalization of *AQP4* in response to binding of AQP4-IgG has been demonstrated in *in vitro* models, but it was not found in mouse cortical astrocyte cultures or mouse brain *in vivo*.[13]

Finally, because transient hyperCKemia can precede CNS demyelinating attacks, CK elevations could herald impending CNS events.[3,4,8] We propose that it may be prudent to add CK values to blood tests in NMO patients with myalgias or proximal weakness to evaluate for evidence of muscle involvement. High values may prompt consideration of more aggressive immune therapies to protect the CNS. With better surveillance with CK measurements, the incidence of hyperCKemia in NMO should be investigated further.

Abbreviations

AQP4	aquaporin-4
BN/PAGE	blue-native polyacrylamide gel electrophoresis
BSA	bovine serum albumin
CK	creatine phosphokinase
CNS	central nervous system
CSF	cerebrospinal fluid
EMG	electromyography
H&E	Hematoxylin and Eosin stains
Ig	immunoglobulin
MRC	Medical Research Council
MRI	magnetic resonance imaging
NADH	nicotinamide adenine dinucleotide
NMO	neuromyelitisoptica
OAP	orthogonal arrays of particles
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

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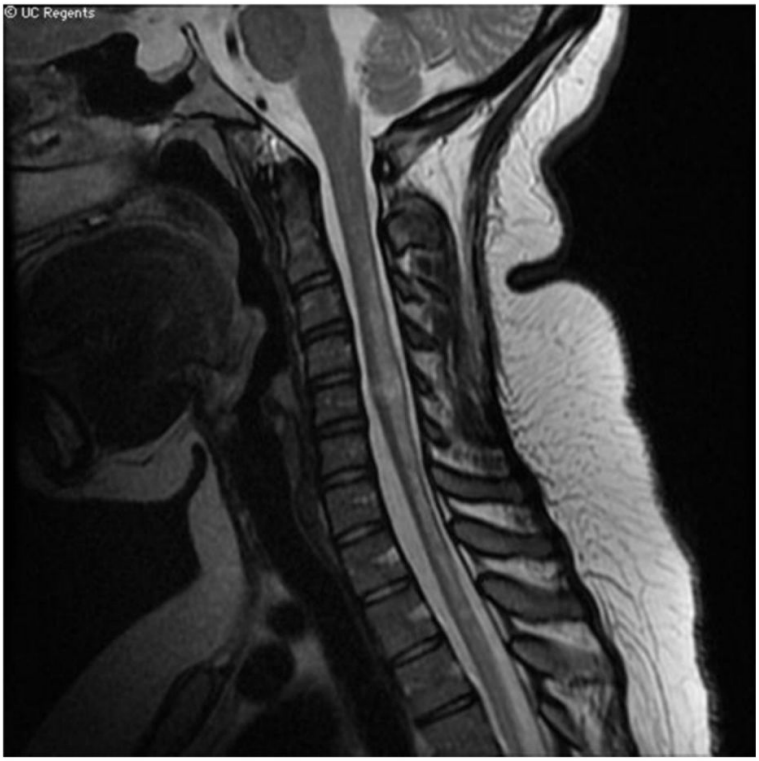

FIGURE 1.

Sagittal T2-weighted image of the cervical spine (patient 2) demonstrating T2 prolongation in the cord from C3 to T2. There was focal enhancement at the C5 level (not shown).

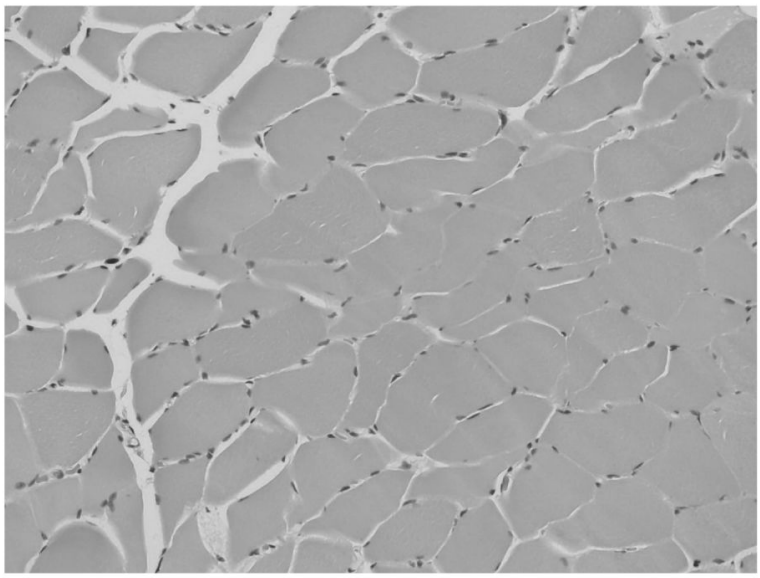

FIGURE 2.

H&E stain (patient 1). There is mild variation of muscle fiber diameter. Notably, no degenerating or regenerating fibers are seen, and there is no inflammatory infiltrate.

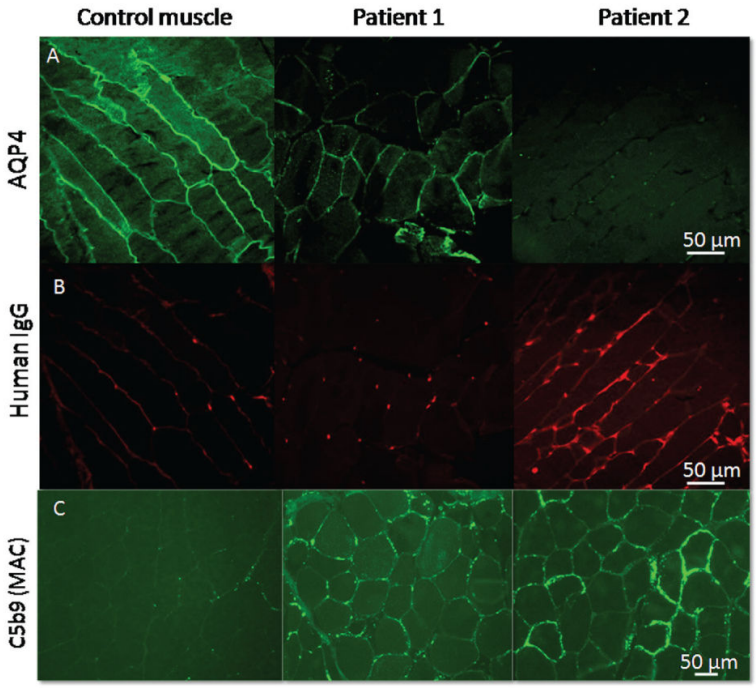

FIGURE 3.

(A) *AQP4* Immunofluorescence (top row, green) showing decreased *AQP4* immunoreactivity on the muscle plasma membrane. **(B)** Human IgG binding detected with antihuman secondary antibody (middle, red), in patient 2. **(C)** C5b9/membrane attack complex immunofluorescence (bottom, green) showing sarcolemmal staining in both patients.

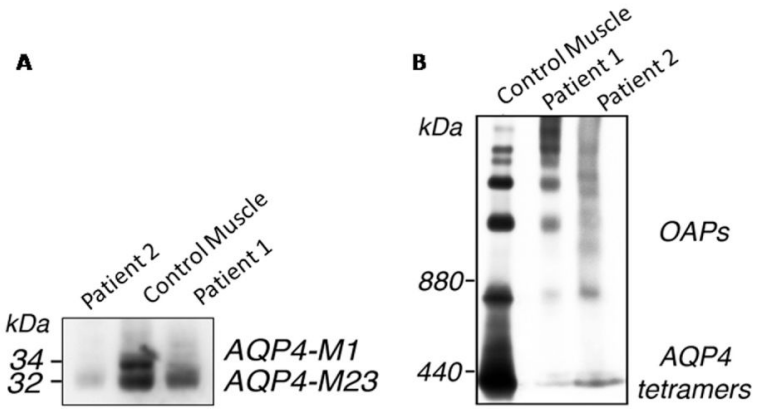

FIGURE 4.

AQP4 immunoblot following SDS-PAGE **(A)** showing reduced *AQP4* protein expression and BN-PAGE **(B)** of indicated muscle homogenates demonstrating relatively greater *AQP4* supramolecular aggregates in the NMO patient biopsies.