

Asymptomatic or Minimally Symptomatic HyperCKemia: Histopathologic Correlates

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Abstract

Background: Persistent creatine kinase elevation is occasionally encountered in subjects without any clinical manifestation of a neuromuscular disorder or any condition known to be associated with increased serum CK levels. It is still unresolved whether extensive investigations and specifically a muscle biopsy should be performed in clinically normal individuals with elevated CK levels.

Objective: To study the muscle pathology of patients with asymptomatic or minimally symptomatic hyperCKemia.

Methods: The clinical and laboratory data of patients with persistent hyperCKemia and normal neurologic examination were reviewed and their muscle biopsies evaluated.

Results: The study group included 40 patients aged 7–67 years; the male to female ratio was 3:1. Nineteen patients were completely asymptomatic, 20 had mild non-specific myalgia, and 1 had muscle cramps. Electromyography was performed in 27 patients and showed myopathic changes in 7 (26%). Abnormal muscle biopsy findings (e.g., increased variation in fiber size, increased number of central nuclei, and occasional degenerating fibers) were detected in 22 of the 40 patients (55%). No fat or glycogen accumulation was detected. Immunohistochemistry demonstrated abnormal dystrophin staining in 3 patients (8%), resembling the pathologic changes of Becker muscular dystrophy. No abnormal findings were detected on immunohistochemical staining for merosin, dysferlin, caveolin 3, or alpha and gamma sarcoglycans. The EMG findings did not correlate with the pathologic findings.

Conclusions: Abnormal muscle biopsies were found in 55% of patients with asymptomatic or minimally symptomatic hyperCKemia. Specific diagnosis of muscular dystrophy, however, was possible in only 8% of the patients.

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is occasionally encountered in subjects without any clinical manifestation of a neuromuscular disorder or any condition known to be associated with increased serum CK levels. The term “idiopathic hyperCKemia” was first coined in 1980 by Rowland et al. [11] to describe a condition characterized by unexplained persistent serum CK elevation unaccompanied by neurologic abnormalities. Most patients with unexplained persistent hyperCKemia follow a benign course. Nevertheless, elevated CK levels may precede the clinical expression of ominous neuromuscular diseases in a small percentage of patients [12–14]. With the advent of laboratory technology, it is now known that CK elevation may also result from subclinical muscle disorders, such as mutations in the caveolin-3 gene or carrier status for mutations in the dystrophin gene [15–18]. The need for extensive ancillary investigations, particularly muscle biopsy, in clinically normal individuals with elevated serum CK levels remains an unresolved issue. We performed a retrospective evaluation of muscle biopsies in individuals with “idiopathic hyperCKemia” in order to characterize the clinical features and assess the prevalence of muscle pathology in this disorder.

Patients and Methods

Patients

We identified 40 patients who had been referred for muscle biopsy because of idiopathic hyperCKemia between 1994 and 2004 and who met the following criteria: a) Persistent elevation of serum CK levels (> 260 U/L) in at least three separate measurements at rest (i.e., no muscle exercise for 1 week); the elevated CK fraction was of the muscle type. b) Lack of symptoms or only mild non-specific myalgia. c) No history of exercise intolerance or rhabdomyolysis. d) Normal neurologic examination. e) No family history of a neuromuscular disorder. f) Absence of conditions known to be associated with hyperCKemia, such as thyroid or other endocrine diseases, use of drugs (e.g., statins), metabolic, hematologic or neoplastic disorders, alcoholism, or malignant hyperthermia.

A needle electromyography examination had been performed in 27 subjects and a forearm ischemic test in 12.

Chronic elevation of serum creatine kinase levels is a common manifestation of neuromuscular disorders [1]. It can also occur in association with various other conditions such as exposure to drugs and toxins, alcoholism, physical exercise, muscle trauma, hyperthermia, pregnancy, malignancies, endocrine disorders, and infections [2–10]. Persistent CK elevation

CK = creatine kinase
EMG = electromyography

Histopathologic evaluation

Fresh muscle tissue was snap-frozen with isopentane, quenched in liquid nitrogen to a temperature of -150°C for 1 minute and then stored at -80°C . Samples were cut to a width of 6 μm . The following histologic and histochemical staining procedures were performed in all specimens: hematoxylin and eosin, modified Gomori trichrome, NADH dehydrogenase, periodic-acid Schiff, Sudan black, cytochrome c oxidase, succinate dehydrogenase, and ATPase at pH 9.4, 4.3 and 4.6.

Immunohistochemistry

Immunohistochemical staining for dystrophin 1, 2 and 3, α and γ sarcoglycans and caveolin-3 was performed in all specimens. Staining for merosin and dysferlin was performed in 26 and 20 specimens, respectively. Monoclonal antibodies for dystrophin 1, 2 and 3 (diluted 1:20), dysferlin (diluted 1:20), α and γ sarcoglycans (diluted 1:100) were purchased from Novocastra Laboratories (Newcastle, UK). Monoclonal antibodies for caveolin-3 (diluted 1:100) were purchased from Santa Cruz Biotechnology (USA). The AEC detection kit was purchased from the Ventana Medical System (USA). Immunohistochemical staining was performed on a Ventana Nexes automatic stainer instrument. The staining is based on an indirect biotin-avidin system resulting in dark red precipitates at the site of antigens. All stained slides were compared to normal controls.

Results

Patients

Forty patients (30 males and 10 females) with asymptomatic or mildly symptomatic hyperCKemia who underwent muscle biopsy during the period 1994–2004 were identified. Their mean age was 27 (range 7–67 years): 11 subjects were younger than 20 years, 24 subjects were aged between 20 and 40, and 5 were older than 40. Serum CK levels in at least three separate measurements after rest ranged between 265 and 8000 U/L. Twenty-one patients had serum CK levels > 1000 U/L and 19 had CK < 1000 U/L. Four subjects (10%) had familial hyperCKemia without evidence of a neuromuscular disorder.

Nineteen individuals (48%) were completely asymptomatic, 20 (50%) had mild non-specific myalgias, and 1 (3%) complained of muscle cramps. None evidenced exercise intolerance or myoglobinuria and they all had normal neurologic examination with normal muscle strength and muscle mass. The study patients' characteristics are summarized in Table 1.

Electrophysiologic studies

An EMG examination was performed in 27 subjects: 9 were asymptomatic and 18 were mildly symptomatic. It was normal in 19 (70%), and showed mild myopathic changes without spontaneous activity in 7 (26%). One patient (4%) had occasional fasciculations with normal motor unit potentials. Five (63%) of the 8 subjects with abnormal EMG had a normal muscle biopsy. The EMG was normal in 6 of 9 (67%) of the asymptomatic subjects, compared to 13 of 18 (72%) of the mildly symptomatic subjects.

Table 1. Characteristics of the subjects with hyperCKemia

Patients	Group 1 (CK < 1000 U/L)	Group 2 (CK > 1000 U/L)	Total
N	19	21	40
Gender			
Males	15	15	30
Females	4	6	10
Symptoms			
Asymptomatic	7 (37%)	12 (57%)	19 (48%)
Symptomatic	10 (63%)	7 (43%)	21 (52%)
EMG			
Normal	9/12 (75%)	10/15 (67%)	9/27 (70%)
Myopathic	2/12 (17%)		7/27 (26%)
Fasciculations	1/12 (8%)	5/15 (33%)	1/23 (4%)
Muscle biopsy			
Normal	9 (47%)	9 (43%)	18 (45%)
Abnormal	10 (53%)	12 (57%)	22 (55%)

Table 2. Histopathologic findings of 22 subjects with abnormal muscle biopsy findings

Findings	No. (%)
Central nuclei	19 (86%)
Regenerative fibers	15 (68%)
Increased fiber size variability	11 (50%)
Necrotic fibers	6 (27%)
Angulated fibers	3 (14%)
Splitting	2 (9%)
Nuclear bags	1 (4.5%)
Type I fiber atrophy	1 (4.5%)
Type II predominance	1 (4.5%)

Forearm ischemic test

The forearm ischemic test for lactate and ammonia was performed in 12/40 (30%) and was normal in all.

Muscle histopathology

Routine histochemical studies demonstrated abnormal findings in 22 of the 40 patients (55%) and normal in 18 (45%). The most common abnormalities were internal nuclei, regenerative fibers, and increased fiber size variability. Other changes included necrotic fibers, angulated fibers, fiber type predominance and atrophy [Figure 1]. Table 2 summarizes the histopathologic findings.

The muscle biopsy was abnormal in 12 of 21 (57%) of the mildly symptomatic patients, compared to 9 of 19 (47%) of the asymptomatic patients. Serum CK levels did not correlate with the biopsy findings: 10 of 19 (53%) of the patients with CK < 1000 U/L had abnormal muscle biopsy, compared to 12 of 21 (57%) of the patients with CK > 1000 U/L. The muscle biopsy was abnormal in 5 of 8 (63%) with an abnormal EMG.

Immunohistochemistry

Immunohistochemical staining for dystrophin 1, 2 and 3 was performed in all 40 subjects and demonstrated abnormal dystrophin expression in 3 (8%) [Figure 2]. Dystrophin was

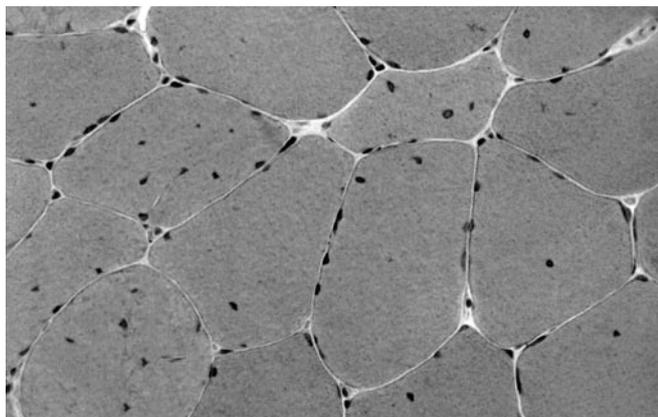


Figure 1. Biopsy sample (H&E staining) taken from the quadriceps muscle of a 42 year old man with hyperCKemia, showing myopathic changes: central nuclei, increased fiber size variability, and one large round fiber.

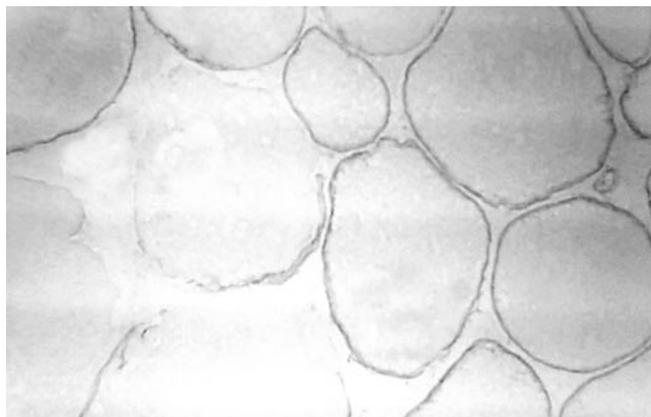


Figure 2. Biopsy sample (staining for dystrophin) taken from the quadriceps muscle of a 58 year old man with hyperCKemia. Normally the sarcolemma of all fibers is homogeneously stained. This specimen shows partial staining consistent with dystrophinopathy.

homogeneously reduced in two patients (12 and 58 year old males), and expressed in a mosaic pattern in one (a 16 year old female). All three were asymptomatic, and the abnormal CK levels (6000 U/L in the boy, 600 U/L in the girl, and 2300 U/L in the adult male) were detected incidentally. Immunohistochemical studies for sarcoglycan α and γ (all subjects), caveolin-3 (all subjects), dysferlin (26 subjects), and merosin (24 subjects) were all normal.

Discussion

In this study, muscle biopsy was abnormal in 55% of the patients with idiopathic hyperCKemia. Non-specific myopathic changes were the most common abnormalities. A definitive etiologic diagnosis for the elevated CK levels could be made in only 8% of patients, all of whom had dystrophinopathy.

Muscle biopsy showed only non-specific myopathic changes in 48% (19/40) of our patients. The most common abnormalities were increased amounts of internal nuclei, regenerative fibers, and increased fiber size variability. Less common changes included necrotic and angulated fibers, splitting, fiber I atrophy, fiber II predominance, and nuclear bags. Several authors have described neurogenic changes in idiopathic hyperCKemia [12,19]. None of our patients had a neurogenic biopsy pattern. The nuclear bags that were found in one patient were insufficient to establish the diagnosis of a neurogenic process: this patient also had myopathic changes, so we assume that the nuclear bags were non-specific changes that can also sometimes occur in myopathies.

Three of our patients (8%) had dystrophinopathy. Since two of them were children, it is possible that they were still in the preclinical stage of the disease. The third individual was a 50 year old man. All three patients were asymptomatic. Dystrophinopathies are known to have quite a variable phenotypical expression, varying from very severe to almost asymptomatic [20].

Mutations in the caveolin-3 gene were reported in familial asymptomatic hyperCKemia [15–17]. Immunohistochemistry for

caveolin was performed in all 40 patients in our series and showed normal caveolin expression in all. Caveolin was also normal in another large series of patients with idiopathic hyperCKemia [19]. Therefore, the yield of immunohistochemistry for caveolin is apparently low in idiopathic hyperCKemia.

The absence of dysferlin is known to cause muscular dystrophy or distal myopathy [21–23]. In one study, dysferlinopathy was found in a 16 year old girl in whom hyperCKemia was the presenting symptom of muscular dystrophy. Dysferlin expression was normal in our patients, as was immunohistochemistry for α and γ sarcoglycans. We routinely study only α and γ sarcoglycans since the absence of β and δ sarcoglycans causes a secondary reduction in the expression of the other sarcoglycans [24,25].

Our results showed that an abnormal EMG was not correlated to abnormal muscle biopsy. Of the 8 patients with abnormal EMG, 5 (62%) had a normal biopsy. However, the number of patients is too small to arrive at a definitive conclusion. Serum CK levels did not correlate with biopsy findings in our study: both subgroups (CK < 1000 U/L and > 1000 U/L) had an almost equal percentage of abnormal muscle biopsies.

Elevation of the serum CK activity indicates that the integrity of the muscle cell membrane has been affected. The commonly encountered abnormalities of increased numbers of internal nuclei, regenerative fibers, and increased fiber size variability may reflect a slow necrotizing process that is not severe enough to produce dystrophy. The heterogeneity of the pathologic findings in these patients and the fact that persistent hyperCKemia can be either familial or sporadic, however, suggest that hyperCKemia is not a single clinical entity, but rather a reservoir of various neuromuscular conditions that have yet to be elucidated.

In conclusion, the results of our study demonstrate that muscle biopsy is frequently abnormal in patients with persistent hyperCKemia. Most abnormalities are non-specific, but may reflect membrane instability with occasional necrosis. The yield of muscle biopsy in confirming a definite diagnosis is relatively

low. More studies focusing on the genetic and biochemical mechanisms are needed to improve our understanding of the pathogenesis of this condition.

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