

Muscle Biopsy and In Vitro Contracture Test in Subjects with Idiopathic HyperCKemia

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Background: Persistent high creatine kinase (CK) levels may reflect underlying subclinical myopathies. In most cases, pathogenesis is unknown and clinical management is unclear. Though clinically asymptomatic, these subjects are potentially susceptible to malignant hyperthermia.

Methods: The authors analyzed 37 subjects with persistent elevation of CK without significant weakness or other neurologic symptoms. Neurologic examination was performed according to manual muscle testing. Muscle biopsy and the *in vitro* contracture test were performed in all subjects.

Results: Twenty-three subjects (51.1%) were completely asymptomatic. The others had minor symptoms such as occasional cramps (11 subjects, 24.4%), fatigue (5 subjects, 11.1%), a combination of cramps and fatigue (5 subjects, 11.1%), and muscle pain (1 case, 2.2%). Muscle biopsy enabled precise diagnosis in 3 cases and was normal in 3 cases. The more frequent changes were variation in fiber size (31.1%), a combination of nuclear internalization and variation in fiber size (26.6%), nuclear internalization (6.6%), minor mitochondrial changes (4.4%), and neurogenic atrophy (4.4%). Immunocytochemical analysis was normal in all patients. *In vitro* contracture testing detected one malignant hyperthermia-susceptible and one malignant hyperthermia-equivocal subject.

Conclusions: The evidence of malignant hyperthermia susceptibility by *in vitro* contracture test seems to be relatively infrequent among subjects with idiopathic hyperCKemia, but the incidence of true malignant hyperthermia in idiopathic hyperCKemia is unknown. Muscle biopsy should be considered a useful, though not very sensitive, diagnostic tool in idiopathic hyperCKemia, because it enables potentially treatable disorders, such as inflammatory myopathies, to be discovered. No uniform morphologic finding typical of idiopathic hyperCKemia or malignant hyperthermia susceptibility was identified by muscle biopsy.

THE term *idiopathic hyperCKemia* (IH) is used to define a persistently elevated serum concentration of creatine kinase (CK) without weakness or other significant neuromuscular symptoms.¹ Asymptomatic IH is more common in males and may occur at any age. A mild transient increase in serum concentrations of CK may be caused by muscle injury and exercise and may occur in normal people. Persistently abnormal levels of CK may be the

only apparent feature of endocrine or metabolic myopathies, limb-girdle muscular dystrophy, and other hereditary or acquired myopathies, particularly in their early stages.¹ Weglinski *et al.*² studied a series of subjects with IH and found positive contracture test results in 49%, but no correlation emerged between CK levels, histologic changes, and malignant hyperthermia (MH) susceptibility. In a recent review of individuals with IH,³ the authors found a high percentage of autosomal dominant cases and no specific findings. In most cases, pathogenesis is unknown and clinical management is unclear. Though clinically asymptomatic, IH patients could be susceptible to MH. Here, we report a series of 37 Italian subjects with IH, in which biopsy studies including the *in vitro* contracture test (IVCT) were performed.

Materials and Methods

According to Italian regulation regarding observational and retrospective studies, a simple notification of the hospital ethics committee is sufficient. We have regularly notified the hospital ethics committee of the plan of the current study.

In this retrospective study, we included subjects who came to our observation in the past 4 yr with elevated serum concentrations of CK discovered incidentally (reference range, 70–170 UI/l). The CK test was performed because of minimal neuromuscular symptoms (cramps, myalgia, and others), in approximately half the subjects; in the others, the CK test was part of routine serum chemistry. We examined 37 patients (26 males and 11 females ranging in age from 11 to 69 yr). Persistent hyperCKemia was determined by four monthly measurements at rest (no exercise for 1 week) before performing muscle biopsy. Maximum and average concentrations of CK are reported in table 1. Neurologic evaluation was performed by manual muscle testing (British Medical Research Council Motor Grading Scale). No subject had a history of anesthesiologic complications or a family history of neuromuscular disorders or anesthesiologic incidents. Occasional causes of hyperCKemia, such as malignancies, drug and alcohol abuse, thyroid and parathyroid disorders, infections, and hematologic diseases, were excluded. None of the subjects were using statins or other drugs potentially capable of inducing hyperCKemia. All subjects underwent routine serum chemistry, including serum myoglobin. Clinical and laboratory investigation of various family members did not reveal any familial cases of

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Table 1. Summary of the Results of the Clinical, Laboratory, and Muscle Biopsy Studies for Each Individual

	Sex/Age, yr	IVCT	CK	Symptomatology	Muscle Biopsy	Diagnosis
1	F/11	MHN	m.v. 312 UI/l, a.v. 180 UI/l	Asymptomatic	Slight variation in fiber size	
2	M/15	MHN	m.v. 780 UI/l, a.v. 330 UI/l	Asymptomatic	Slight variation in fiber size	
3	F/16	MHN	m.v. 890 UI/l, 4,110 after exercise, a.v. 486 UI/l	Occasional cramps	No change	
4	M/18	MHN	m.v. 209 UI/l, 1,048 after exercise, a.v. 620 UI/l	Asymptomatic	Variation in fiber size, 5% internal nuclei	
5	M/20	MHN	m.v. 5,732 UI/l, a.v. 1,486 UI/l	Fatigue	8% internal nuclei, slight variation in fiber size	
6	M/21	MHN	m.v. 3,376 UI/l, a.v. 1,540 UI/l	Asymptomatic	Slight variation in fiber size, 7% internal nuclei	
7	M/22	MHN	m.v. 387 UI/l, a.v. 286 UI/l	Occasional cramps, fatigue	Slight variation in fiber size	
8	M/22	MHN	m.v. 330 UI/l, a.v. 186 UI/l	Occasional cramps	Slight neurogenic atrophy	
9	M/23	MHN	m.v. 3,800 UI/l, a.v. 986 UI/l	Asymptomatic	No change	
10	F/25	MHN	m.v. 491 UI/l, a.v. 235 UI/l	Asymptomatic	No change	
11	M/26	MHN	m.v. 398 UI/l, 1,177 after exercise, a.v. 245 UI/l	Occasional cramps	Variation in fiber size, 5% internal nuclei	
12	F/27	MHN	m.v. 271 UI/l, a.v. 230 UI/l	Asymptomatic	Slight variation in fiber size	
13	M/27	MHN	m.v. 269 UI/l, a.v. 228 UI/l	Asymptomatic	Slight variation in fiber size	
14	M/27	MHN	m.v. 400 UI/l, a.v. 220 UI/l	Fatigue	Variation in fiber size, 17% internal nuclei	
15	F/27	MHN	m.v. 605 UI/l, a.v. 307 UI/l	Asymptomatic	5% internal nuclei	
16	M/28	MHN	m.v. 800 UI/l, 1,700 after exercise, a.v. 586 UI/l	Occasional cramps, fatigue	Variation in fiber size, 6% internal nuclei	
17	M/29	MHS	m.v. 407 UI/l, a.v. 208 UI/l	Asymptomatic	Slight variation in fiber size	
18	M/30	MHN	m.v. 216 UI/l, a.v. 204 UI/l	Asymptomatic	Slight variation in fiber size	
19	M/31	MHN	m.v. 569 UI/l, a.v. 386 UI/l	Asymptomatic	13% internal nuclei, slight variation in fiber size	
20	M/35	MHN	m.v. 578 UI/l, a.v. 283 UI/l	Occasional cramps	Slight variation in fiber size	
21	M/35	MHN	m.v. 538 UI/l, a.v. 286 UI/l	Asymptomatic	Slight increase in sudanophilic lipids	
22	M/35	MHN	m.v. 1,740 UI/l, a.v. 466 UI/l	Asymptomatic	Variation in fiber size, nuclear internalization 8%	Dystrophinopathy
23	F/37	MHN	m.v. 20,000 UI/l, a.v. 3,486 UI/l	Asymptomatic	Slight variation in fiber size	
24	M/36	MHN	m.v. 527 UI/l, a.v. 220 UI/l	Occasional cramps	Slight variation in fiber size, prevalence fiber I type	
25	M/41	MHN	m.v. 259 UI/l, a.v. 205 UI/l	Occasional cramps	Slight variation in fiber size	
26	F/44	MHN	m.v. 269 UI/l, a.v. 216 UI/l	Fatigue	5% internal nuclei, variation in fiber size	
27	M/46	MHN	m.v. 730 UI/l, a.v. 486 UI/l	Occasional cramps	Core-like lesions	
28	M/49	MHN	m.v. 579 UI/l, a.v. 432 UI/l	Nocturnal cramps	11% internal nuclei, variation in fiber size	
29	M/50	MHN	m.v. 479 UI/l, a.v. 289 UI/l	Occasional cramps, fatigue	Slight variation in fiber size, prevalence fiber II type	
30	M/55	MHN	m.v. 544 UI/l, a.v. 376 UI/l	Fatigue	Slight neurogenic atrophy	
31	M/55	MHN	m.v. 544 UI/l, a.v. 333 UI/l	Occasional cramps, fatigue	Internal nuclei 5%, slight variation in fiber size	
32	F/59	MHN	m.v. 506 UI/l, a.v. 275 UI/l	Occasional cramps	Variation in fiber size, 5% internal nuclei	
33	M/62	MHN	m.v. 358 UI/l, 987 after exercise, a.v. 323 UI/l	Asymptomatic	Slight variation in fiber size variation, 0.5% cox-negative fibers, some moth-eaten fibers	
34	F/63	MHN	m.v. 450 UI/l, a.v. 255 UI/l	Asymptomatic	0.5% cox-negative fibers	
35	F/65	MHN	m.v. 543 UI/l, a.v. 384 UI/l	Occasional cramps, fatigue	Minor mitochondrial changes	
36	M/65	MHN	m.v. 8,200 UI/l, a.v. 2996 UI/l	Muscle pain	Endomysial infiltrates, rare necrosis, regeneration	Inflammation
37	F/69	MHE	m.v. 473 UI/l, a.v. 254 UI/l	Fatigue	Central core changes	Central core changes

a.v. = average value; CK = creatine kinase; IVCT = *in vitro* contracture test; MHE = malignant hyperthermia equivocal; MHN = malignant hyperthermia negative; MNS = malignant hyperthermia susceptible; m.v. = maximum value.

IH. To discover subclinical myopathies, electromyography of the bilateral deltoid, biceps brachii, anterior tibial, and rectus femoris muscles was routinely performed in all cases. Open muscle biopsy from the quadriceps femoris was performed in all subjects. The specimens were examined with routine stains (hematoxylin and eosin; modified Gomori trichrome; cytochrome *c* oxidase; succinate dehydrogenase; NADH dehydrogenase; adenosine triphosphatase at pH 10.4, 4.6, and 4.3; periodic acid-Schiff; and Sudan) and reactions for myoadenylate deaminase, myophosphorylase, and phosphofructokinase. Immunocytochemical analysis was performed using antibodies against dystrophin (Dys1, 2, and 3); α , β , δ , and γ sarcoglycans; caveolin; dysferlin; emerin; and merosin. Muscle specimens were also processed by standard methods for ultrastructural examination.

In vitro contracture testing was performed according to the caffeine-halothane contracture protocol of the European Malignant Hyperpyrexia Group on fresh specimens of vastus lateralis muscle.⁴ Briefly, patients were considered MH susceptible (MHS) if a contracture of at least 2 mN occurred after exposure to 2 mM caffeine and 2% halothane, MH equivocal if muscle specimens contracted in response to only one of the triggering agents, and MH negative if contracture response was normal to both drugs.

Mutation screening of RYR1 gene was performed with denaturing high-performance liquid chromatography (WAVE 3500 HT system; Transgenomic, Omaha, NE) and sequencing analysis (ABI PRISM 310 genetic analyzer; Applied Biosystems [Applied], Foster City, CA) of the three regions of the gene in which mutations result to be clustered (exons 2-17, 39-46, and 90-104).

Statistical Analysis

Descriptive statistics were calculated using Microsoft Excel 2003 for Windows (Redmond, WA). Event rates were reported as percentages with 95% confidence intervals.

Results

Muscle biopsy was completely normal in 3 cases (8.1%) [95% confidence interval, 3.2-13]. In 11 cases (29.7%) [26-33.4], we observed variation in fiber size, and in another 10 cases (27%) [23.1-30.9], we observed a combination of variability in fiber size and nuclear internalization. In 3 subjects (8.1%) [3.2-13], a variation in fiber size was associated with variable findings such as type II and I predominance, and minor mitochondrial changes. One subject (2.7%) [-2.5 to 7.8] showed nuclear internalization, 2 subjects (5.4%) [0.4-10.4] had minor mitochondrial changes, and 2 (5.4%) [0.4-10.4] had neurogenic atrophy. Two subjects (5.4%) [0.4-10.4] showed variable findings such as core-like lesions and slight lipid increase. In 3 subjects (8.1%) [3.2-13], a diagnosis was reached (inflammatory myopathy, central

core changes without clinical manifestation, and dystrophinopathy) (table 1). Immunocytochemical analysis was normal in all subjects (except the patient with dystrophinopathy). Ultrastructural examination did not show any significant lesions. The subject identified as MHS showed frequent vesiculation of the T-tubule system.

Seventeen individuals (45.9%) [95% confidence interval, 43.0-48.8] were completely asymptomatic. The others had minor symptoms such as occasional cramps (8 subjects, 21.6%) [17.5-25.7], fatigue (5 subjects, 13.5%) [8.9-18.1], a combination of cramps and fatigue (5 subjects, 13.5%) [8.9-18.1], and muscle pain (1 subject, 2.7%) [-2.5 to 7.8].

One subject (2.7%) [-2.5 to 7.8] was identified as MHS by IVCT, and the subject with central core changes was MH equivocal.

Molecular analysis of RYR1 gene in the MHS subject revealed two novel nucleotide changes: c.7085 A>G of exon 44 and c.13513 G>C of exon 92, predicted to cause p.E2362G and p.D4505H amino acid changes, respectively.

Electromyography yielded normal results in 22 cases and myopathic results in 15 subjects.

Discussion

Muscle biopsy analysis is regarded as the principal tool for screening asymptomatic subjects with IH. Our initial screening allowed us to make a precise diagnosis in 3 (8.1%) of 37 patients. The percentage of final diagnoses has varied in the few series reported so far.⁵⁻⁷ In a recent study on "clinically normal subjects" with chronic hyperCKemia,⁵ a diagnosis was achieved in 55% of cases. However, the authors described severe biopsy findings but not clinical details and included subjects with serum CK levels greater than 500 U/l, which may partly explain the difference. Differences between the various series of subjects are presumably due to different inclusion criteria. It is important to define the concept of "asymptomatic subject." For example, whereas occasional myalgias or cramps may be found in normal people, persistent myalgias or cramps, mostly after exercise, may be significant symptoms even in the absence of local weakness. Extensive biochemical studies are advisable in cases with positive biopsy findings.

At the time of evaluation, the remaining 34 patients fulfilling diagnostic criteria for IH were clinically stable and did not report muscle weakness. Histopathologic analysis of muscle biopsies revealed nonspecific myopathic lesions. The most frequent alterations were variation in fiber size, followed by nuclear internalization and minor mitochondrial changes, or a variable combination of these changes. We found no relationship among biopsy findings, serum levels of CK, and age of patients. Even minor symptoms, observed in some patients, were not related to serum levels of CK or biopsy changes. The treatment of patients with persistently

elevated serum levels of CK, usually without clinical symptoms, is a difficult and puzzling problem for anesthesiologists, neurologists, and physicians.^{2,8,9} In the current study, only three subjects had completely normal muscle biopsies; clinical management of subjects with IH should therefore include a clinical and laboratory follow-up because apparently normal patients may develop neuromuscular disorders in the future. IH probably includes different undiscovered neuromuscular disorders. Our study suggests that with current diagnostics, muscle biopsy does not reach an etiologic diagnosis in most subjects with persistently increased serum levels of CK but without significant myopathic symptoms. Although increased serum levels of CK are a valid reason to screen for underlying neuromuscular disorders, it is debatable to consider this finding predictive of MH susceptibility, unless the subject belongs to a family with a history of anesthesiologic complications.^{8,10-12} Subjects with IH may be part of the clinical spectrum of MH, but subclinical myopathies may give false-positive IVCT results. No certain correlation has been reported between serum levels of CK and MH susceptibility.^{7,8,10-13}

A remarkable finding of the current study was the rarity of MH susceptibility among subjects with elevated serum levels of CK; of 37 patients undergoing ICVT, only 1 (2.7%) was MHS. The percentage of MH susceptibility among individuals with IH is not clear; reports in the literature show variable percentages.^{2,9,14} However, in large cohorts of patients with a high percentage of subjects positive to ICVT, patient selection was not reported. Sometimes they were enrolled on the basis of anesthesiologic complications in the family. To date, there is no agreement about what, if any, histopathologic findings are characteristic of MH susceptibility. In a series of 83 cases selected on the basis of anesthesiologic incidents, myopathic findings such as variation in fiber size, nuclear centralization, and fiber necrosis were found in MHS subjects, but no changes were detected in MH-negative subjects. In other studies, no histomorphologic differences were found between MHS and MH-negative patients. In the current study, the MHS and MH-equivocal subjects did not show any peculiar histopathologic findings with respect to other patients with IH.^{2,7} Ultrastructural abnormalities involving the T-tubule system, such as unusual swelling and vesiculation, have been reported. Our findings are in accord with these reports. The equivocal response of a subject with central core changes may be due to variation in concentration of halothane and caffeine during the procedure, especially if the equivocal response regards halothane. Furthermore, it is possible that not all muscles of a single subject react in the same way to trigger agents.

The relatively low percentage of MH susceptibility among patients with IH may therefore not be a surprising finding. Some authors suggest that CK levels can only

be used to screen for MH susceptibility if associated with anesthesiologic complications in the family.⁹ Patients with susceptibility to MH and normal CK serum concentrations of CK have been reported.² We suggest that muscle biopsy study and IVCT are useful for clinical treatment of subjects with IH; MHS subjects or those with subclinical myopathy should avoid trigger anesthetics, whereas subjects with IH should only avoid depolarizing muscle relaxants such as succinylcholine. No subject with IH should be treated with statins or other drugs that may trigger rhabdomyolysis. The patients of the current series undergo clinical evaluation and assay of serum CK every 6 months. None have yet shown muscle weakness or other additional symptoms; no further diagnosis has been reached, and CK values continue to be elevated but below their maximum values.

We suggest that muscle biopsy is still a useful, though not very sensitive, diagnostic tool in IH, because it enables potentially treatable disorders, such as inflammatory myopathies, to be discovered. MH susceptibility seems to be low among subjects with IH; serum concentrations of CK should not be regarded as a screening index for MH. No morphologic finding typical of IH or MH susceptibility was identified by muscle biopsy.

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