CASE REPORT

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Carnitine deficiency

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Abstract

We present the case of a female patient, aged 12 years, with fatigability and exertional myalgias, progressively developed within the last two years. Negative family history, as well as negative personal medical history, were found. At physical examination, short stature, proximal muscle weakness and mild hepatomegaly were noted. Urine ketones level was slightly decreased, serum transaminases, creatine kinase and lactate dehydrogenase levels were increased. Electromyographical examination showed a myopathic non-specific pattern. Deltoid muscle biopsy revealed: small, clear vesicles are present on Hematoxylin–Eosin and modified Gömöri trichrome stains; modified Gömöri trichrome stain also revealed muscle fibers (especially type I of muscle fibers) having mild to moderate mitochondrial proliferation (red rim and speckled sarcoplasm). The lipid storage has been well demonstrated by Sudan Black stain, which revealed small lipid droplets in type I muscle fibers. Abnormal internal architecture with a punctate pattern was showed by adenine dinucleotide tetrazolium reductase and succinate dehydrogenase stains. Electron microscopy showed small inter-myofibrillar accumulations of round, amorphous, homogeneous acellular substances that are not membrane bounded. These features indicate that these are neutral fat (lipid) droplets. Subsarcolemmal accumulations of mitochondria were also revealed. The differential diagnosis of this case is discussed, and the up to date general data concerning carnitine deficiency are presented. The aim of our case-report is to emphasize the role of muscle biopsy in carnitine deficiency, as well as to remind the necessity of keeping in mind such metabolic disorders when doing the differential diagnostic of a muscular weakness.

Keywords: carnitine, deficiency, diagnostic, muscle biopsy.

Introduction

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) is an essential cofactor for the oxidation of fatty acids by mitochondria. It serves to carry long-chain fatty acids in the form of their acyl-carnitine esters across the barrier of the inner mitochondrial membrane before β-oxidation. Biologic effects of low carnitine levels may not be clinically significant until they reach less than 10–20% of normal. Carnitine deficiency may be primary or secondary [1, 2].

Primary carnitine is an autosomal recessive disorder of fatty acid oxidation caused by defective carnitine transport. The lack of the plasma membrane carnitine transporter results in urinary carnitine wasting and in decreased intracellular carnitine accumulation. Patients may present as infants with non-ketotic hypoglycemia, hypotonia, Reye syndrome or sudden infant death or later in life with cardiomyopathy (characteristically dilated) or muscle weakness. Causative mutations in a gene called *OCTN2* are responsible for this condition [1, 2].

Carnitine deficiency limited to the muscle is observed in myopathic carnitine deficiency with severe reduction in muscle carnitine levels. The basic biochemical defect has not been identified.

Secondary carnitine deficiency, which manifests with a decrease of carnitine levels in plasma or tissues, may be associated with genetically determined metabolic conditions, acquired medical conditions or iatrogenic states [3–5].

Carnitine deficiency is known as a "rare disease", even no studies have estimated the incidence of primary carnitine deficiency in Romania; in Australia, for example, the incidence was recently estimated to be between 1:37 000–1:100 000 newborns [3, 4, 6].

Patient and Methods

A 12-year-old female patient presented with fatigability and exertional myalgias, progressively developed within the last two years.

For positive and differential diagnose purposes, a complete anamnesis, together with physical examination and the following tests were performed:

- Blood glucose level: frequently decreased in carnitine deficiency;
- Urine ketones level: absence or low amounts of ketones in the urine in primary carnitine deficiency, as well as in other defects in the carnitine cycle or fatty acid oxidation;

▪ Blood ammonia level: can be moderately elevated, especially in primary carnitine deficiency and particularly if the child has a presentation similar to that of Reye syndrome;

▪ Serum transaminases:

– Usually moderately elevated in primary carnitine deficiency;

– In some defects of the carnitine cycle that cause secondary carnitine deficiency (e.g., CPT-II deficiency), a hepatocardiomuscular form can present with liver involvement;

– Other fatty acid oxidation disorders, such as long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency, can present with liver involvement) [3–6].

▪ Blood chemistry panel: may show evidence of metabolic acidosis;

▪ Blood uric acid level: hyperuricemia may be present in carnitine deficiency because carnitine competes for renal tubular excretion;

▪ Serum creatine kinase and lactate dehydrogenase levels: increased in primary carnitine deficiency and in fatty acid oxidation disorders;

▪ Serum lactate level: elevated in respiratory chain defects or in LCHAD deficiency.

▪Coagulation tests: prolonged prothrombin time may be found;

▪ Fasting test:

– Patient undergone a controlled and prolonged fast under strict medical supervision;

– Blood samples were obtained at regular intervals to measure glucose, ketone bodies, and free fatty acids;

– Fasting may be continued in children for up to 24 hours, unless blood glucose drops to less than 3 mmol/L;

– An inadequate production of ketones with a high free fatty acid-to-ketone bodies ratio suggests a defect in long-chain fatty acid oxidation [4, 6].

▪ Brain imaging studies may show cystic lesions in glutaric aciduria type II or basal ganglia involvement in mitochondrial disorders that may be associated with secondary carnitine deficiency [1, 2];

▪ Electrocardiogram:

– The ECG reveals left ventricular hypertrophy and peaked T-waves in primary carnitine deficiency;

– Cardiac arrhythmias can be observed in translocase deficiency and in the lethal neonatal form of carnitine palmitoyltransferase II (CPT-II) deficiency [5, 6].

▪ Electromyography: used to confirm the myopathic nature of muscle weakness;

▪ Muscle biopsy: necessary to confirm the diagnosis of some conditions that may cause secondary carnitine deficiency (e.g., respiratory chain defect) or to rule out other myopaties).

Results

Negative family history, as well as negative personal medical history, were found.

At physical examination, short stature, proximal muscle weakness and mild hepatomegaly were noted.

▪ Blood glucose level was normal;

▪ Urine ketones level was slightly decreased, 80% of the inferior limit of laboratory normal range;

▪ Blood ammonia level was normal;

▪ Serum transaminases were moderately elevated: two times over the superior limit of laboratory normal range;

▪ Blood chemistry panel showed no evidence of metabolic acidosis;

▪ Blood uric acid level was normal;

▪ Serum creatine kinase level was increased 10 times over the superior limit of laboratory normal range;

▪ Serum lactate dehydrogenase level was increased six times over the superior limit of laboratory normal range;

▪ Serum lactate level was normal;

▪ Fasting test: inadequate production of ketones with a high free fatty acid-to-ketone bodies ratio (=4.3);

▪ Brain imaging studies showed no pathological sign;

▪ Electrocardiogram revealed rare atrial extrasystoles;

▪ Electromyographical examination showed a myopathic non-specific pattern;

▪ Deltoid muscle biopsy revealed:

– Muscular fragment with normal architecture, but containing numerous normal size muscle fibers with multiple very small vesicles. Vesicles were present in both histoenzymatic types of muscle fibers.

– Muscle fibers without vesicles had normal external shape and normal internal structure but had reduced size (compared to normal fiber size for patient's age), often being even atrophic and sometimes forming small groups.

– Small, clear vesicles are present on Hematoxylin–Eosin and modified Gömöri trichrome stains (Figure 1).

– Modified Gömöri trichrome stain also revealed muscle fibers (especially type I of muscle fibers) having mild to moderate mitochondrial proliferation (red rim and speckled sarcoplasm) (Figure 1).

– The lipid storage can be well demonstrated by Sudan black stain, which revealed small lipid droplets in type I muscle fibers (Figure 2).

– Abnormal internal architecture with a punctate pattern was showed by adenine dinucleotide tetrazolium reductase and succinate dehydrogenase stains.

– Some other non-specific changes were observed too: a moderate variability of the fiber size without a specific topography and without changes in fiber shape excepting two fibers with a more or less angular contour, some fibers with 1–2 internal nuclei, a splitting fiber. The proportion of type 2 fibers was severely reduced.

– Several fibers had no staining for cytochrome oxidase.

– Electron microscopy showed small intermyofibrillar accumulations of round, amorphous, homogeneous acellular substances that are not membrane bounded. These features indicate that these are neutral fat (lipid) droplets. Subsarcolemmal accumulations of mithocondria were also revealed (Figure 3).

– Immunohistochemical studies for dystrophins, sarcoglycans, and dystroglycans was also performed, revealing normal results.

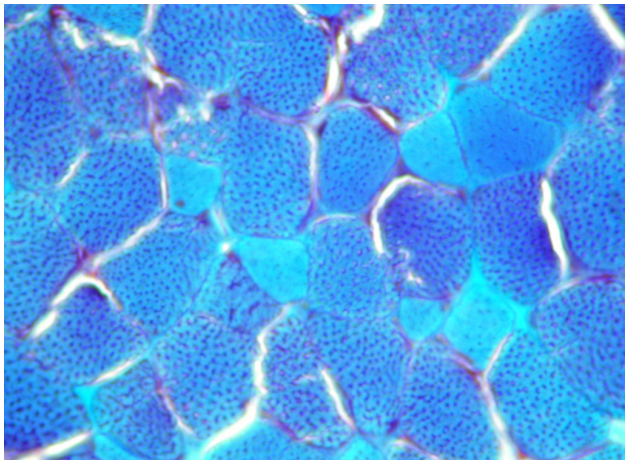

Figure 1 – ***Muscle fibers with mild to moderate mitochondrial proliferation (red rim and speckled sarcoplasm). Intermyofibrillar lipid small accumulations (vesicles) are present (modified Gömöri trichrome stain, ob. ×40).***

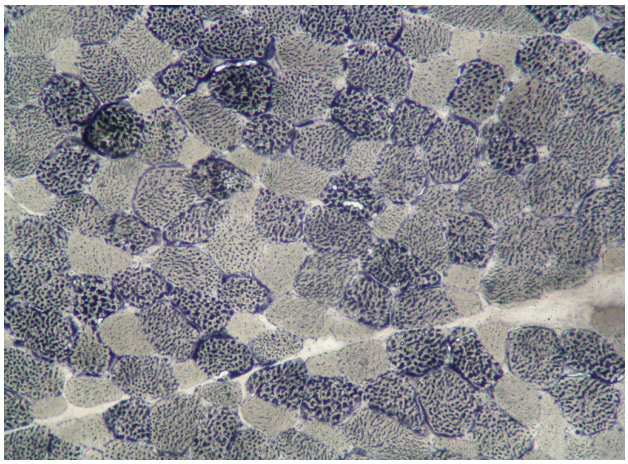

Figure 2 – ***The lipid storage can be well demonstrated by Sudan Black stain which revealed small lipid droplets in type I muscle fibers (ob. ×40).***

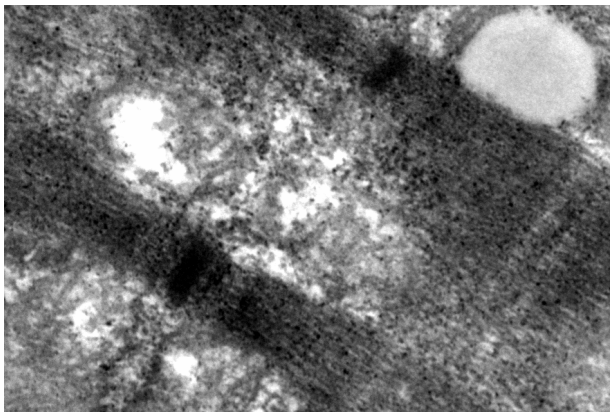

Figure 3 – ***Electron microscopy showed small intermyofibrillar accumulations of round, amorphous, homogeneous acellular substance that are not membrane bounded. These features indicate that these are neutral fat (lipid) droplets, ×20 000.***

Discussion

Carnitine deficiency has been observed in children with urea cycle defects (e.g., ornithine transcarbamylase deficiency, carbamoyl phosphate synthetase deficiency). Whether carnitine deficiency is related to the primary metabolic defect, to the concomitant liver disease observed in the initial presentation, or to benzoate therapy is unclear. Carnitine deficiency is also observed in disorders of the mitochondrial respiratory chain, such as cytochrome *c* oxidase deficiency, in which the ATP depletion may compromise the energy-dependent carnitine uptake. An interference with carnitine transport occurs in tissues, including renal reabsorption, which explains the low plasma and tissue levels in these patients [5, 6].

Other inborn errors of metabolism or genetic disorders may cause secondary carnitine deficiency because of impairment of carnitine biosynthesis secondary to increased urinary losses of lysine, which occurs in lysinuric protein intolerance. Increased urinary loss of carnitine associated with Fanconi syndrome may be observed in syndromes such as cystinosis or Lowe syndrome (i.e., X-linked oculocerebrorenal syndrome) [5–7].

Acquired medical conditions may affect carnitine homeostasis. Cirrhosis or chronic renal failure may impair the biosynthesis of carnitine. Diets with low carnitine content (e.g., lacto-ovo-vegetarian diet) or malabsorption syndromes may cause secondary carnitine deficiency. It may also be observed in conditions of increased catabolism present in patients with critical illness. Increased losses of carnitine in the urine, which occur in renal tubular acidosis or Fanconi syndrome, may cause secondary carnitine deficiency. Iatrogenic causes of secondary carnitine deficiency include several drugs associated with secondary carnitine deficiency (e.g., valproate, pivampicillin, emetine, zidovudine), but none of them was taken by our patient [2, 6, 7].

The personal history of our patient helped us to eliminate from the beginning periodic paralyses and the toxic myopathies from the differential diagnosis of our patient. His age and clinical data as well as the morphological aspect of his muscle biopsy sustain the diagnostic of myopathy due to the carnitine deficiency.

Muscle fibers containing multiple vacuoles characterizing sarcotubular myopathy have a different aspect than those of our case, as well as peroxisomal vacuoles in Lafora disease, which can be recognized on cryostat sections as multiple basophilic dense dots, only the largest ones looking like vacuoles.

Molecular genetic tests could not be performed for our patient that is why for the differential diagnosis we had to consider only his clinical and existing paraclinical data. In this given context, the muscle biopsy was of major importance for a correct diagnostic, which made possible an appropriate therapeutic approach and a correct prognosis.

Conclusions

Carnitine deficiency is a rare metabolic disorder of fatty acid oxidation with different types of presentation that have been observed within an individual family. Diet may contribute to the pathogenesis of this disorder.

Delayed diagnosis has been reported as common in cases with slowly progressive symptomatology, as was our patient.

The aim of our case report is to emphasize the role of muscle biopsy in this difficult diagnose processes, as well as to remind the necessity of keeping in mind such metabolic disorders when doing the differential diagnostic of a muscular weakness.

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