ABSTRACT

Carnitine palmitoyltransferase II deficiency (CPT II) is an autosomal recessive inherited disorder of long-chain fatty acid oxidation in the mitochondrial matrix, resulting in an inability to utilize fat for energy in cells. The most frequent myopathic form occurs in young adults and is associated with recurrent episodes of exercise-induced rhabdomyolysis. The myopathic form is caused by the Ser113Leu mutation of the CPT II gene. Rarely, massive rhabdomyolysis could be complicated by acute kidney injury (AKI), cardiomyopathy, and respiratory insufficiency.

We present a case of an 18-year old male with myalgia, muscular weakness, and dark-colored urine after prolonged exercise and a recent mild SARS-CoV-2 infection. Massive rhabdomyolysis was diagnosed with markedly increased serum concentrations of myoglobin and creatine kinase, with normal kidney function. The patient experienced two similar episodes in the years 2017 and 2018, with rhabdomyolysis and AKI treated with hemodialysis. After excluding autoimmune and infectious diseases as causes of recurrent rhabdomyolysis, the patient was genetically tested and Ser113Leu mutation of the CPT II gene was confirmed.

When a patient presents with myalgia and dark-colored urine triggered by minor physical activities, genetic testing for possible CPT II deficiency should be initiated. The SARS-CoV-2 infection could be a factor that triggers the occurrence of rhabdomyolysis and aggravates the severity of the attack in patients with CPT II deficiency.

Keywords: carnitine palmitoyl transferase II deficiency; hereditary disease; rhabdomyolysis; SARS-CoV-2 infection

INTRODUCTION

Carnitine palmitoyltransferase (CPT) I and II are enzymes that play a crucial role in the “shuttling” of long-chain fatty acids into the mitochondrial matrix for subsequent β-oxidation and energy production in cells [1-3]. Deficiency of CPT II is the most common inherited disorder of long-chain fatty acid oxidation, resulting in an inability of the cells to utilize fat for energy during
stress or prolonged fasting [2]. This condition is presented as lethal neonatal form, severe infantile hepatocardiomuscular form, and the most frequent adult-onset myopathic form [4].

It is inherited as an autosomal recessive disorder, and partial CPT II deficiency with an autosomal dominant inheritance pattern has also been reported. The first symptoms of the myopathic form most commonly occur in the first two decades of life [4]. The attacks of recurrent rhabdomyolysis and myoglobinuria triggered by prolonged exercise, fasting, infection, exposure to cold or heat, emotional stress, and drugs are the main clinical features of the adult-onset myopathic form [1-4]. The SARS-CoV-2 infection and COVID-19 vaccine have also been associated with rhabdomyolysis in patients with CPT II deficiency or a similar metabolic disorder [5, 6]. In approximately 7% of cases with CPT II deficiency, massive rhabdomyolysis could be complicated by acute kidney injury, respiratory failure, cardiac arrhythmias, cardiomyopathy, or other life-threatening events [7].

CASE PRESENTATION

We report a case of 18-year old male with CPT II deficiency who was admitted to the hospital with myalgia, generalized muscular weakness, dark coloring of urine, and nausea with vomiting. Two days before the first symptoms, the patient had intensive physical exercise, walking more than 9 km. The patient was not taking any drugs (ibuprofen, diazepam, or valproic acid) which are associated with increased risk for rhabdomyolysis. Three weeks prior, the patient had been diagnosed with the SARS-CoV-2 infection with mild symptoms. According to the official protocols for treatment of the COVID-19 infection, the patient was advised to stay at home in isolation for 10 days.

On admission, the patient’s body temperature was 36.9 °C, heart rate of 100 bpm, blood pressure of 130/70 mmHg, and oxygen saturation at 99%. The patient had muscle tenderness, mostly in the lower limbs. A PCR test for SARS-CoV-2 was negative, with the presence of IgG antibodies (SARS-CoV-2 IgG: 12.26 AU/ml). Evaluation of laboratory parameters demonstrated leukocytosis of 15.8x10⁹/L, with neutrophilic predominance (>88%), elevated serum level of aspartate aminotransferase (AST) 2058 U/L (normal 10-34 U/L), elevated serum level of serum alanine aminotransferase (ALT) 304 U/L (normal 10-45 U/L), elevated serum level of lactate dehydrogenase (LDH) 1847 U/L (normal < 248 U/L), elevated serum level of creatine kinase (CK) 102410 U/L (normal 24-173 U/L), and elevated serum myoglobin

![Graph 1](attachment:image.png)  
**Graph 1.** Distribution of the serum levels of creatine kinase, myoglobin and creatinine, during the hospital treatment of the patient
777 ng/ml (normal < 75.0 ng/ml). Serum levels of creatinine and blood urea nitrogen were within normal limits. Diuresis was greater than 8 L, following an initial 2-days decline in urine output before admission to the hospital. Electrocardiography, echocardiography, and ultrasonography examination of the kidneys were within normal findings. A CT scan of the lungs was performed because of the recent SARS-CoV-2 infection. This scan was also without pathological findings. The patient was treated with extensive fluid replacement. The serum levels of creatine kinase and myoglobin became significantly reduced during the hospital treatment of the patient (Graph 1).

He was discharged from the hospital with a recommendation for high fluid intake and lifestyle modification to prevent further attacks. One month after hospitalization, during regular checkup, the patient was clinically stable, without muscle pain, with a serum level of creatine kinase slightly above the upper normal limit (213 U/L).

The patient had a medical history of two similar episodes in the years 2017 and 2018, with rhabdomyolysis, myoglobinemia, and acute kidney injury treated with hemodialysis. The onset of symptoms occurred after prolonged exercise in sport classes and then enterocolitis. The repetitive rhabdomyolysis with acute kidney injury was highly suspicious for metabolic disorder. Based on the clinical features and supportive laboratory findings, CPT II deficiency was assumed. The diagnosis of CPT II deficiency was established by molecular genetic testing of biallelic pathogenic variants in the CPT II gene. Ser113Leu (c.338C>T) was detected, which is associated with the adult myopathic form of the hereditary CPT II deficiency. Subsequently, a genetic analysis revealed that the patient’s brother was also homozygous for the Ser113Leu mutation of the CPT II gene, but without symptoms that required medical attention. Both parents were not genetically tested, and they have never experienced any similar symptoms.

**MUTATION ANALYSIS OF CPT II GENE**

Single-gene mutation analysis was performed after informed consent was obtained. DNA was collected from the patient and automatically extracted from whole blood using the MagCore® Genomic DNA Whole Blood Kits (MagCore ® HF16 Plus Automated Extraction System; ATRiDA B.V., Amersfoort, Netherlands). Three pairs of primers were designed to amplify three fragments of the CPT II gene (RefSeq: NM_000098), covering exon 1 (299bp), 3 (231 bp), and 4 (564bp) (forward primer sequences - Ex 1: 5’-ACTCCCAGAACTCCCTGCCTTG-3’; Ex3: 5’-CCTCGCCATGAACCTAAAAA-3’; Ex4: 5’-CCCATTAAGGACCCTTGCCA-3’), reverse primer sequences: Ex 1: 5’-CGGGTTCACTAGAGTAGCTCA-3’; Ex 3: 5’-TTCATTATGGAGGGCTCTGG-3’; Ex 4: 5’-GCCTCAGAGCACCCTTTG-3’). PCR products were labeled with the Applied Biosystems BigDye terminator version 3.1 sequencing kit (Thermo Fisher Scientific, Waltham, MA, USA) and purified with the BigDyeXTerminator Purification Kit (Applied Biosystems). The sequence products were run on an automated ABI 3500XL gene analyzer (Thermo Fisher Scientific), and the results were analyzed with Sequencing Analysis v5.4 software (Thermo Fisher Scientific).

**DISCUSSION**

Inherited metabolic diseases, as carnitine palmitoyltransferase deficiency, are the most common causes of recurrent rhabdomyolysis and myoglobinuria in young people [4]. Carnitine palmitoyltransferase is present in two sub-forms: CPT I and CPT II. CPT I is located at the outer mitochondrial membrane and facilitates the formation of long-chain acylcarnitine and coenzyme A. CPT II is located on the inner mitochondrial membrane in cells and catalyzes the reverse reaction of producing acyl-coenzyme A (acyl-CoA) [2]. The acyl-CoA that is produced is available for subsequent β-oxidation and energy production in the mitochondrial matrix of the cell [1-3]. Inherited disorders of the CPT II enzyme follow an autosomal recessive mode of inheritance. More than 25 mutations of the CPT II gene have been described in the literature, mostly missense mutations in exons 1, 3, 4, and 5 [4]. Ser113Leu is the most common mutation in adult-myopathic form, with a frequency higher than 76% [8-10]. It is the most frequent mutation among the European population, with male predominance (>70%) [8-10]. It remains unclear whether the male predominance reported in the studies by Martin MA et al. and Blanc PL...
et al. is due to sex-related differences in exercise activities, hormonal, or X-chromosomal modifier genes that influence CPT activity [11, 12]. The phenotypic variability in siblings and among patients with the same gene mutation is well documented [4]. The younger brother of the case patient has the same Ser113Leu mutation, but he has never experienced an attack of rhabdomyolysis. This phenotypic variability suggests that other environmental and/or genetic factors might influence disease severity [4]. This mutation has already been described in two native patients: a 22-year old male with rhabdomyolysis and AKI treated with hemodialysis, and a 20-year old male with a more severe form of the disease with AKI, hepatic lesion, respiratory insufficiency, and cardiomyopathy with volume overload, treated plasmapheresis and noninvasive ventilation support [13, 14].

Joshi PR et al. reported that myalgia followed by muscle weakness, fatigue/heaviness, feeling of muscle stiffness and muscle cramps were the most common symptoms in their study of 13 patients with CPT II deficiency [9]. Leg muscles (thigh and calves) and proximal arms, as the most active muscle groups, were affected in more than 92% of the patients, while back/trunk and face muscles were involved in more than half of evaluated patients [9]. Pathohistological evaluation of muscle biopsies in patients with CPT II deficiency revealed unspecific myopathic changes, including atrophic muscle fibers and slight lipid accumulation [10]. Prolonged exercise was a trigger for attacks in more than 90% of the patients with CPT II deficiency. It is considered that physical activity lasting for 15 to 60 minutes could provoke symptomatic rhabdomyolysis in more than half of the patients [9]. In only 15% to 18% of the patients, rhabdomyolysis may be complicated with myoglobinuria and AKI that requires treatment with hemodialysis [9, 10]. Ivin N et al. concluded that the patients with CK levels higher than 110.660 U/L on admission would probably need some form of organ support (including hemodialysis), versus a group of patients with lower CK levels [3].

Electron microscopic examination of renal specimens performed by Kanekoa H et al. revealed acute tubular necrosis of the proximal tubule cells and intraluminal myoglobin casts [15]. These myoglobin casts could cause AKI, predominantly non oliguric AKI, and/or impaired tubular concentrating mechanism with polyuria [15, 16]. Most patients will become asymptomatic one week after initiation of the attack, with a clinically symptom-free period between the attacks [1-3].

Rhabdomyolysis has been recognized as one of the extrapulmonary manifestations of COVID-19 [17, 18]. Murillo F et al. described a patient with severe pneumonia caused by SARS-CoV-2 who required mechanical ventilation and developed myocarditis and rhabdomyolysis without progression to AKI, two weeks after the first symptoms [18]. Many authors theorized that an exaggerated immunological reaction (cytokine storm) in the COVID-19 infection could lead to a depletion of cellular energy, rupture of myocyte membrane with an increase of intracellular calcium, and subsequent cellular death [17, 18]. Severe rhabdomyolysis with AKI and the fatal outcome was documented in a 23-year-old female with a similar metabolic disorder (deficiency of Long-chain L-3 hydroxyacyl-CoA dehydrogenase) [5]. Rhabdomyolysis was noted in a patient with CPT II deficiency, five hours after receiving the COVID-19 Vaccine (AstraZeneca) [6]. Hydroxychloroquine and oseltamivir were used in the treatment of the COVID-19 infection as well as Triton X–100 as a component of the AstraZeneca vaccine. These have been reported as possible trigger factors for rhabdomyolysis [6, 17].

The clinical phenotype in our case-patient was characteristic for the adult myopathic form of CPT II deficiency, starting at childhood and presenting as recurrent rhabdomyolysis complicated with AKI. The recent SARS-CoV-2 infection was probably one of the triggers that facilitated the occurrence of rhabdomyolysis and aggravated the severity of the last attack.

In conclusion, the frequency of CPT II deficiency has been underestimated and many patients with CPT II deficiency and a milder form of rhabdomyolysis remain undiagnosed [19]. Therefore, when a patient presents with myalgia and dark-colored urine triggered by minor physical activities or infection, genetic testing for possible CPT II deficiency should be initiated. Early diagnosis of this condition is extremely important because it provides an opportunity for those patients to modify their everyday lifestyle to avoid recurrent attacks of rhabdomyolysis and to prevent the development of complications. There is no definitive treatment for CPT II deficiency, but early intensive hydration to increase kidney perfusion prevents the intratubular precipitation of myoglobin [9, 20]. The SARS-CoV-2 infection should not be ruled out as a trigger for the occurrence of rhabdomyolysis and thus aggravating the severity of the attack in patients with CPT II deficiency. These patients should
have close medical monitoring during and after the SARS-CoV-2 infection and Covid-19 vaccine, with the protocol being 21 days in isolation. Ongoing surveillance is required to evaluate the incidence of CPT II deficiency in all patients with SARS-CoV-2 infection presenting with rhabdomyolysis.

Data availability
The data related to this case report are not publicly available because they could compromise the privacy of the patient. The data relevant for this case will be provided by corresponding author, Irena Rambabova-Bushljetik, upon request.

Conflict of Interest Statement
All authors have nothing to disclose. The results presented in this paper have not been published previously.

Consent for publication
Yes

Funding statement
This study did not receive any funding in any form.

REFERENCES

Резиме
РАБДОМИОЛИЗА АСОЦИРАНА СО СКОРЕШНА ИНФЕКЦИЈА НА SARS-COV-2 КАЈ ПАЦИЕНТ СО ДЕФИЦИТ НА ЕНЗИМОТ КАРНИТИНПАЛМИТОИЛ ТРАНСФЕРАЗА II

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Дефицитот на ензимот карнитин палмитоилтрансфераза II (КПТ II) е автозомно рецесивно наследно нарушување на оксидацијата на масните киселини со долги синџири, што резултира со неможност тие да се искористат за добивање енергија во клетките. Најчестата миопатска форма на заболувањето се појавува кај младите возрасни и се поврзува со повторувачките епизоди на рабдомиолиза индуцирани од напор или од инфекција. Миопатската форма е предизвикана од мутацијата Ser113Leu во генот за КПТ II. Масивната рабдомиолиза може да биде асоцирана со акутно бубрежно оштетување (АБО), кардиомиопатија и респираторна инсуфициенција.

Даваме приказ на 18-годишно момче со миалгија, мускулна слабост и темнопребоена урина, кои следеле по пролонгирана умерена физичка активност и скорешна лесна форма на инфекција на SARS-CoV-2. Кај пациентот беше дијагностицирана масивна рабдомиолиза со високи вредности на моцните киселини, кои се поврзуваат со повторувачките епизоди на рабдомиолиза индуцирани од напор или од инфекција. Миопатската форма е предизвикана од мутацијата Ser113Leu во генот за КПТ II. Масивната рабдомиолиза може да биде асоцирана со акутно бубрежно оштетување (АБО), кардиомиопатија и респираторна инсуфициенција.

Ключни зборови: карнитин палмитоилтрансфераза II дефицит, наследна болест; рабдомиолиза, инфекција на SARS-CoV-2