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The value of fasting in the diagnosis of medium-chain acyl-CoA dehydrogenase deficiency

O valor do jejum no diagnóstico da deficiência de acil-CoA desidrogenase de cadeia média

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ABSTRACT

Female patient carrier of medium-chain acyl-CoA dehydrogenase deficiency (MCADD) with recurrent clinical episodes of hypoglycemia and altered level of consciousness, presented changes in blood acylcarnitine profile by tandem mass spectrometry and in the urinary organic acid analysis by gas chromatography/mass spectrometry (GC/MS). This case demonstrates the importance of fasting prior biological sample collection (when possible) when MCADD is suspected, and emphasizes that the time/momentum of biological sample collection is crucial to diagnosis, considering the possibility that MCADD is underdiagnosed in Brazil.

Key words: mitochondrial β-oxidation defects; MCADD; fasting; acylcarnitines; organic acids.

RESUMO

Paciente portadora de deficiência de acil-CoA desidrogenase de cadeia média (MCADD), com episódios clínicos recorrentes de hipoglicemia e alteração de consciência, apresentou alterações no perfil de acilcarnitinas em sangue por espectrometria de massas em tandem e na análise de ácidos orgânicos urinários por cromatografia gasosa acoplada à espectrometria de massa. Este caso demonstra a importância da coleta de amostra biológica em jejum (se possível) quando há suspeita de MCADD e ressalta que o tempo/momento de coleta da amostra biológica é importante para o diagnóstico, considerando a possibilidade de a MCADD ser subdiagnosticada no Brasil.

Unitermos: *defeitos da betaoxidação mitocondrial; MCADD; jejum; acilcarnitinas; ácidos orgânicos.*

RESUMEN

Paciente portadora de deficiencia de acil-CoA deshidrogenasa de cadena media (MCADD) con episodios clínicos recurrentes de hipoglucemia y alteración de consciencia presentó mudanzas en el perfil de acilcarnitinas en la sangre con técnicas de espectrometría de masas en tándem y en el análisis de ácidos orgánicos urinarios mediante cromatografía de gases acoplada a espectrometría de masas. Este caso demuestra la importancia de la toma de muestras biológicas en ayunas (se posible) cuando se sospecha de MCADD y destaca que el tiempo/momento de extracción de la muestra biológica es valioso para el diagnóstico, considerando la posibilidad de que la MCADD es subdiagnosticada en Brasil.

Palabras clave: *defectos de la beta-oxidación mitocondrial; MCADD; ayuno; acilcarnitinas; ácidos orgánicos.*

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INTRODUCTION

Medium chain acyl-CoA dehydrogenase deficiency (MCADD) is an autosomal recessive disease caused by mutations in the acyl-CoA dehydrogenase gene, C-4 to C-12 straight chain (ACADM) that encodes this enzyme[1-4]. Most patients are homozygous for a single missense mutation at exon 11of the *ACADM* gene, the c.985A>G transition translated into replacing the lysine residue by a glutamic acid at position 304 in the mature protein (p.Lys304Glu)[5-10]. It is of considerable clinical heterogeneity and high mortality rate. The most common symptoms include recurrent hypoketotic hypoglycemia, lethargy, vomiting, acute liver dysfunction, Reye-like syndrome episodes, seizures and coma, sudden death may also occur[1-10].

Individuals with MCADD may remain clinically and laboratory asymptomatic until the occurrence of a prolonged period of fasting – often associated with a concomitant virus infection, more common in children – or after intense physical exercise, which is common in adolescents and adults. These scenarios trigger metabolic stress and, consequently, patient decompensation[1-10].

Treatment for MCADD consists of providing appropriate calorie intake and avoiding fasting, catabolism situations and infectious episodes. The prevention of metabolic decompensation in a patient diagnosed with MCADD involves the establishment of an early diagnosis, preferably presymptomatic. The establishment of a carbohydrate-enriched lipid-poor diet supplemented with oral carnitine may also prevent acute episodes[1-6]. Once the disease is diagnosed and therapeutic measures are properly implemented, recurrence of metabolic crisis will be rare; therefore, the prognosis of this pathology is very favorable[1-10].

In a condition suggestive of MCADD, a differential diagnosis is necessary, as this condition presents clinical signs common to other beta-oxidation disorders. Medium chain fatty acid dehydrogenase (MCAD) deficiency is biologically associated with change in the free fatty acids in plasma profile and urine organic acids[1-6, 8-14]. Therefore, initial tests include analysis of the acylcarnitine profile in blood using tandem mass spectrometry (MS/MS), analysis of urine organic acids using gas chromatography coupled to mass spectrometry (GC/MS) and analysis of acylglycines in urine using GC/MS. Confirmatory tests are composed of *ACADM* gene mutation screening by deoxyribonucleic acid (DNA) analysis and additional biochemical genetics testing, such as assays for enzymatic activity of MCAD – leukocytes, fibroblasts or other tissues –, as well as cellular studies of global oxidation in fibroblasts[1, 4, 8-15].

In the present work, we describe the results of the investigation of a suspected case of mitochondrial fatty acid beta-oxidation disorders, specifically MCADD, in a patient with intermittent manifestations at three stages: by analysis of urine organic acids using gas chromatography, by the measurement of acylcarnitines in blood using MS/MS, and by molecular investigation for mutation in *ACADM* gene, stressing the importance of the appropriate time of biological sample collection for laboratory investigation, in order to promote a better knowledge in the investigation of this pathology.

CASE REPORT

Female patient, 1 year and 7 months of age, was referred for investigation of inborn errors of metabolism (IEM) during hospitalization due to recurrent clinical episodes complaining of vomiting, drowsiness and hypoglycemia (**Table**), through the EIM Brazil Network. At the first examination, urine organic acids were investigated using gas chromatography coupled to mass spectrometry (GC/MS)[11] and acylcarnitines and amino acids in

TABLE – Patient clinical data and hospitalization history

Hospitalizations (age)	Clinical data
At birth	Date of birth: 05 June, 2015 Birth weight: 2860 g; weight at discharge: 2710 g; Apgar 9/9 Teenage mother (14 years old); four prenatal visits GA: 38 weeks + 2 days for 26 weeks ECHO Negative serology Cesarean section for active genital herpes No report of parental consanguinity
1st hospitalization (67 hours of life)	Hypoactivity HGT 27 mg/dl Neonatal ICU admission Discharge at 7th of life No record of hypoglycemia during hospitalization

2nd hospitalization (9 months)	Vomiting Sensory lowering Hypoactive and with mild but vigorous breathing effort, but afebrile Very slow perfusion, thin wrists Received 2 SF push 0.9% 20 ml/kg HGT 39 mg/dl Sepsis screening and ceftriaxone initiation Transferred to the pediatric ICU CSF normal; CK 1141 U/l; CKMB 35 U/l; troponin 39 ng/ml Head CT scan unchanged EEG with left diffuse slowing, phenobarbital initiation She was discharged after 23 days with diagnosis of sepsis resolved, with no record of complications during hospitalization
3rd hospitalization (1 year and 4 months)	Vomiting complaints Sensory lowering History of hypoglycemia
4th hospitalization (1 year and 7 months)	Febrile, bradypnea, bradycardia, with HGT 18 mg/dl Received PPV and mask until ventilation recovered Received 5% glycated serum 2 ml/kg, push SF 20 ml/kg Sepsis screening: normal tests results Abdominal ultrasound with no changes Brain MRI unchanged
	Endocrine and genetic consulting
	Endocrine assessment: normal test cortisol/low GH, but isolated deficiency would not justify severe hypoglycemia *Glucose: 44 mg/dl; insulin: 1 mU/l* *Normal arterial blood gas* *Lactate 0.7 mg/dl* *Free fatty acids 1.61 mMol/l* *Glucagon test (GH up to 1.79 and cortisol 18.6)* *Neurological assessment: gradual decrease of phenobarbital* *Tests submitted for screening for IEM*
	Discharge with outpatient return for IEM test results Febrile, bradypnea, bradycardia, with HGT 18 mg/dl
5th hospitalization (1 year and 10 months)	Vomiting and drowsiness complaint FGC, afebrile, paleness, hypoactive, drowsiness, HGT 109 mg/dl CA: RR in two periods of time, NFS, with no heart murmur LA: EDBM with no adventitious noises Innocent abdomen No signs of hemorrhagic suffusion or meningism Well perfused ends Patient follow-up at the service for similar clinical conditions Bone age x-ray: normal MRI normal sella turcica Normal EEG IEM result: medium-chain acyl-CoA dehydrogenase deficiency of fatty acids
6th hospitalization (2 years)	Gastroenteritis Cardiorepiratory arrest Death

GA: gestational age; ECHO: echography; HGT: fasting blood glucose; ICU: intensive care unit; S: sterile saline; IEM: inborn errors of metabolism; CSF: cerebrospinal fluid; CK: creatine kinase; CKMB: MB isoenzyme; CT: computed tomography; EEG: electroencephalogram; PPV: Positive-pressure ventilation; GH: growth hormone; FGC: fair general condition; CA: cardiac auscultation; RR: regular rhythm; NFS: normophonetic sounds; LA: lung auscultation; EDBM: evenly distributed breath murmur; MRI: magnetic resonance imaging.

whole blood filter paper samples using liquid chromatography coupled with tandem mass detector (LC/MS/MS)[12-14]. The result for urine organic acids was normal and the acylcarnitines measure in blood showed an increase in both octanoylcarnitine (C8) and octanoylcarnitine and decanoylcarnitine ratio (C8/C10); there was a decrease in free carnitine (C0) and normal levels of hexanoylcarnitine (C6), decanoylcarnitine (C10) and decenoylcarnitine (C10:1).

In a second phase, at 1 year and 10 months, a new acylcarnitine test in a postprandial blood sample was ordered to confirm the first result, which revealed normal acylcarnitine profile. As the clinical hypothesis of beta-oxidation disorder persisted, days later, a third test for acylcarnitine profile and for urine organic acids screening was ordered in blood samples using cards, and urine sample collected after 4 hours of fasting. A considerable increase of C8, C8/C10 ratio and of C10:1 in the blood acylcarnitine profile and the presence of urinary hexanoylglycine were found. Such laboratory findings were indicative of MCADD.

In order to confirm the biochemical diagnosis of MCADD, molecular analysis of blood filter paper sample was performed[8]. Molecular study of *ACADM* gene showed homozygosis for mutation (c.985A>G) (**Figure**).

After the diagnosis, the patient continued outpatient treatment at the São Lucas Hospital of the Pontifícia Universidade Católica do Rio Grande do Sul (HSL-PUCRS). Unfortunately, a few weeks after the diagnosis of MCAD deficiency was confirmed, she was admitted at the emergency room of another hospital during an episode of acute gastroenteritis, in cardiorespiratory arrest with no response to resuscitation maneuvers, and died.

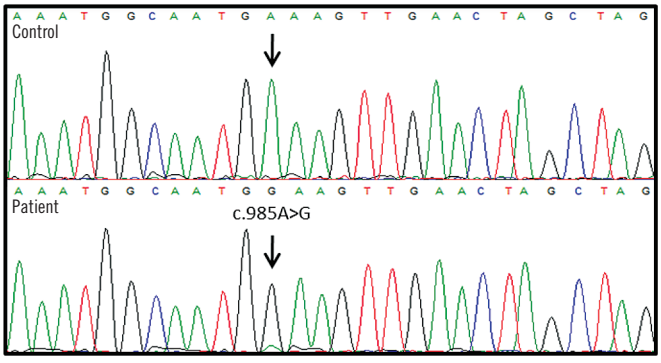

FIGURE – *Genomic DNA sequencing of patient and control. Sequencing analysis of the* ACADM *gene in patient revealed c.985A>G mutation (p.Lys304Glu). Arrows indicate the location of the mutation*

DNA: deoxyribonucleic acid.

DISCUSSION

In several countries, MCAD deficiency is considered the most common cause of mitochondrial fatty acid beta-oxidation disorder, with a prevalence of 1:6500 to 1:7000, comparable to phenylketonuria[14]. It often expresses in the early years of life; the individual presents episodes of metabolic decompensation that may develop neurological sequelae, or even be fatal[1-10]. With the introduction of neonatal screening for MCADD using MS/MS, in addition to monitoring the frequency and incidence of this disease, there was a significant reduction in disease-associated mortality (approximately 30% in the first episode of decompensation) and in morbidity caused by metabolic changes[16, 17]. Therefore, early diagnosis and initiation of treatment have the potential to improve patients prognosis[2].

In Brazil there are no prevalence data of MCADD, only data of heterozygote frequency is available[9]. It is noteworthy that this disease is still underdiagnosed in Brazil, since the diagnosis depends on the measurement of acylcarnitines in blood using MS/MS. Unfortunately, this methodology is not yet widely available in Brazil for patients treated by the Unified Health System [Sistema Único de Saúde (SUS)], nor by public neonatal screening, it is only offered by some private laboratories.

The differential diagnosis for a child with recurrent clinical episodes of vomiting, drowsiness and hypoglycemia, as in this case, is quite broad, including infectious and metabolic causes. Intermediary metabolism diseases usually have nonspecific presentation, such as irritability, hydroelectrolytic imbalance and gastrointestinal disorders, present in numerous common childhood illnesses[17-19]. Many of these cases present an acute course and, when not properly diagnosed and treated, the patient dies without an accurate diagnosis established, which affects the family group as a whole[18-20]. Due to the non-specificity of clinical presentation of many inborn errors of intermediary metabolism, which makes the elaboration of defined hypotheses difficult, the laboratory plays a fundamental role in elucidating the diagnosis[20]. Thus, in a condition suggestive of MCADD, as in the patient described above, a careful investigation into the large group of beta-oxidation disorders is also necessary because we observe common clinical signs, such as vomiting, hypoglycemia, and significant involvement of organs fatty acid-dependent for energy (hypotonia, weakness, and sensory lowering) (Table).

For most mitochondrial beta-oxidation disorders, analysis of urine organic acids using GC/MS will be a sensitive test if samples are collected during acute episodes or when fasting-induced hypoglycemia is present, but it may not be of great diagnostic

value if samples are collected during asymptomatic periods[4, 11-15]. The analysis of the acylcarnitine profile in blood allows detecting the metabolites alteration even when the individuals are clinically stable[4, 12-15]. However, among the hypoglycemic crises, the metabolite profiles may be virtually normal[15], even more if collections are performed after glucose administration or after recovery from a metabolic crisis (characteristic metabolites may have already been eliminated)[1, 15]. There are studies relating changes in the concentration of certain acylcarnitines in postprandial samples[21-23].

The MCADD diagnosis requires considering patient's clinical status (asymptomatic or symptomatic) at the of biological sample collection momentum. MCAD deficiency is, in biochemical basis, associated with an alteration of the free fatty acid profile in plasma and organic acids in urine. Due to enzymatic blockage, medium-chain acyl-CoA esters accumulate in mitochondria and are metabolized by alternative pathways with production of medium-chain dicarboxylic acids (adipic, suberic, sebacic and dodecanedioic acid in the urine) and/or eliminated as acylglycines (urinary hexanoyl- and phenylpropionyl-glycines) and 6-10 carbon acylcarnitines in plasma or on blood filter paper samples, with particular emphasis on C8 and C10:1 levels, essential metabolites in the diagnosis of this pathology[1, 6, 15].

In our patient, the results observed blood samples in card and urine collected at 4 hours of fasting, for acylcarnitines profile (significant increase of C8, C8/C10 ratio and C10:1) and for urinary organic acids screening (presence of hexanoylglycine) were compatible with MCADD. Furthermore, the diagnosis of MCADD was confirmed by molecular analysis of the *ACADM* gene, which showed homozygosis for mutation (c.985A>G), the most frequent for this disease. Homozygosis for mutation (c.985A>G) is credited by some authors as a more severe prognosis of MCADD, however, the occurrence of high heterogeneity of clinical phenotypes (from severe clinical manifestations to absence of symptoms) in patients with the same genotype and belonging to the same family suggests the absence of the clinical phenotype-genotype correlation[4, 7-10].

CONCLUSION

From the clinical point of view, the present case demonstrates the importance of clinical suspicion of an IEM in children with intermittent episodes of hypoglycemia and/or altered levels of consciousness. In addition, the unfavorable outcome of the patient, even after the diagnosis of MCADD, and the therapeutic orientation reinforce the importance of knowledge dissemination among health professionals about the diagnosis and management of beta-oxidation disorders, including MCADD. From the laboratory point of view, this case shows the importance of fasting prior to sample collection (if possible) when a mitochondrial fatty acid beta-oxidation disorder is suspected.

Considering the possibility that MCADD is underdiagnosed in Brazil and is known to be a cause of sudden death in children, it is important to emphasize that the timing/momentum of biological sample collection may be crucial for proper diagnosis and management.

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REFERENCES

1. Roe CR, Ding J. Mitochondrial fatty acid oxidation disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease. New York: McGraw-Hill, New York; 2001; 1909-63.

2. Bastin J. Regulation of mitochondrial fatty acid β-oxidation in human: what can we learn from inborn fatty acid β-oxidation deficiencies? Biochimie. 2014; 96: 113-20. PubMed PMID: 23764392.

3. Derks TG, Boer TS, van Assen A, et al. Neonatal screening for medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in The Netherlands: the importance of enzyme analysis to ascertain true MCAD deficiency. J Inherit Metab Dis. 2008; 31: 88-96. PubMed PMID: 18188679.

4. Waddell L, Wiley V, Carpenter K, et al. B. Medium-chain acyl-CoA dehydrogenase deficiency: genotype-biochemical phenotype correlations. Mol Genet Metab. 2006; 87: 32-9. PubMed PMID: 16291504.

5. Rinaldo P, Matern D, Bennett MJ. Fatty acid oxidation disorders. Annu Rev Physiol. 2002; 64: 477-502. PubMed PMID: 11826276.

6. Wajner M, Amaral AU. Mitochondrial dysfunction in fatty acid oxidation disorders: insights from human and animal studies. Biosci Rep. 2015; 36(1): e00281. PubMed PMID: 26589966.

7. Nichols MJ, Saavedra-Matiz CA, Pass KA, Caggana M. Novel mutations causing medium chain acyl-CoA dehydrogenase deficiency: under-representation of the common c.985A>G mutation in the New York state population. Am J Med Genet A. 2008; 146A: 610-9. PubMed PMID: 18241067.

8. Andresen BS, Bross P, Udvari S, et al. The molecular basis of medium-chain acyl-CoA dehydrogenase (MCAD) deficiency in compound heterozygous patients: is there correlation between genotype and phenotype? Hum Mol Genet. 1997; 6(5): 695-707. PubMed PMID: 9158144.

9. Ferreira AC, Orlandi MPA, Oliveira VC, et al. A985G mutation incidence in the medium-chain acyl-Coa dehydrogenase (MCAD) gene in Brazil. Genet Mol Res. 2009; 8(2): 487-93. PubMed PMID: 19551636.

10. Arnold GL, Saavedra-Matiz CA, Galvin-Parton PA, et al. Lack of genotype-phenotype correlations and outcome in MCAD deficiency diagnosed by newborn screening in New York State. Mol Genet Metab. 2010; 99(3): 263-8. PubMed PMID: 20036593.

11. Sweetman L. Organic acid analysis. In: Hommes FA, editor. Techniques in diagnostic human biochemical genetics: a laboratory manual. New York: Wiley-Liss; 1991. p. 143-5.

12. Rashed M, Bucknall M, Little P, et al. Screening blood spots for inborn errors of metabolism by electrospray tandem mass spectrometry with a microplate batch process and a computer algorithm for automated flagging of abnormal profiles. Clin Chem. 1997; 43: 1127-41. PubMed PMID: 9216448.

13. Schulze A, Lindner M, Kohlmuller D, Olgemoller X, Mayatepek E, Hoffman G. Expanded newborn screening for inborn errors of metabolism by electrospray tandem mass spectrometry ionization tandem mass spectrometry results, outcome and implications. Pediatrics. 2003; 111: 1399-1406. PubMed PMID: 12777559.

14. Chace DH, Hillman SL, van Hove JL, Naylor EW. Rapid diagnosis of MCAD deficiency: quantitative analysis of octanoylcarnitine and other acylcarnitines in newborn blood spots by tandem mass spectrometry. Clin Chem. 1997; 2106-13. PubMed PMID: 9365395.

15. Duran M. Disorders of mitochondrial fatty acid oxidation and ketone body handling. In: Blau N, Duran M, Blaskovics ME, Gibson KM, editors. Physician's guide to the laboratory diagnosis of metabolic diseases. Nova Iorque: Springer; 2003.

16. Ministério da Saúde. Programa Nacional de Triagem Neonatal (PNTN). Dados sobre o Programa de triagem neonatal. Available at: http://portalms.saude.gov.br/acoes-e-programas/programa-nacional-da-triagem-neonatal/dados-sobre-o-programa-nacional-de-triagem-neonatal. [Accessed on: 2018, 5 June].

17. van der Hilst CS, Derks TG, Reijngoud DJ, Smit GP, TenVergert EM. Cost-effectiveness of neonatal screening for medium chain acyl-CoA dehydrogenase deficiency: the homogeneous population of The Netherlands. J Pediatr. 2007; 151(2): 115-20. PubMed PMID: 17643759.

18. Wajner M, Vargas CR, Burin M, Giugliani R, Coelho J. Investigação de erros inatos do metabolismo. Revista HCPA. 2001; (3): 343-60. Available at: https://lume.ufrgs.br/bitstream/handle/10183/163855/000327152.pdf.

19. Sanseverino MT, Wajner M, Giugliani R. Aplicação de um protocolo clínico-laboratorial para identificação de erros inatos do metabolismo em crianças gravemente enfermas. J Pediatr. 2000; 76(5): 375-82. Available at: http://jped.com.br/conteudo/00-76-05-375/port.asp.

20. Dietzen DJ, Rinaldo P, Whitley RJ, et al. National academy of clinical biochemistry laboratory medicine practice guidelines: follow-up testing for metabolic disease identified by expanded newborn screening using tandem mass spectrometry; executive summary. Clin Chem. 2009; 55(9): 1615-26. PubMed PMID: 19574465.

21. Shrestha A, Müllner E, Poutanen K, Mykkänen H, Moazzami AA. Metabolic changes in serum metabolome in response to a meal. Eur J Nutr. 2017; 56(2): 671-81. PubMed PMID: 26658764.

22. Fingerhut R, Arevalo GJS, Baumgartner MR, et al. Postprandial changes of amino acid and acylcarnitine concentrations in dried blood samples. J Inherit Metab Dis. 2010; 33(Suppl 2): S235-9. PubMed PMID: 20652412.

23. Ramos-Roman MA, Sweetman L, Valdez MJ, Parks EJ. Postprandial changes in plasma acylcarnitine concentrations as markers of fatty acid flux in overweight and obesity. Metabolism. 2012; 61(2): 202-12. PubMed PMID: 21820684.

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