Residência RP Pediátrica
Publicação Oficial da Sociedade Brasileira de Pediatria
ISSN-Online: 2236-6814

Submitted on: 05/14/2021
Approved on: 06/13/2021

CASE REPORT

Lysosomal Acid Lipase (LAL) Deficiency: Enzyme Assay on Dried Blood Spot as Diagnostic Tool in a Patient Misdiagnosed as Niemann-Pick type C Disease

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Keywords:
Metabolism.
Inborn errors.
Fatty liver.
Hepatomegaly.
Diagnostic tests.
Routine.

Abstract

Introduction: Lysosomal acid lipase deficiency (LAL-D) is a lysosomal storage disorder involved in cholesterol ester metabolism. It is a poorly understood genetic cause of cirrhosis, dyslipidemia and premature atherosclerotic disease in children and adults. As the manifestations of LAL-D may resemble those observed in other more common diseases, delayed diagnosis is not uncommon. Wolman disease is an early-onset LAL-D phenotype that is usually fatal in the first year of life, but the most common form of the disease may occur at all ages. Recently, enzyme replacement therapy (ERT) for LAL-D has become available. **Case report:** A 12-year-old male patient was referred for hepatomegaly. Initially diagnosed as having Niemann-Pick type C disease (NPC), it did not present confirmatory mutations of this disease. Later, with enzymatic dosage of lysosomal acid lipase on filter paper, the diagnosis was redirected to LAL-D (with confirmation in enzymatic dosage in leukocytes and fibroblasts). **Discussion:** Although LAL-D is considered a rare and uncommon cause of liver disease, recent studies point to a higher prevalence of this disease. Early diagnosis is essential, since specific treatment for this disease is already available. About half of patients with LAL-D die before age 21 in the absence of adequate treatment.

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DOI: 10.25060/residpediatr-2023.v13n3-631

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INTRODUCTION

Lysosomal acid lipase deficiency (LAL) is a hereditary metabolic disease, belonging to a select group of inborn errors of metabolism called lysosomal storage diseases (LSD).[1]

The LAL enzyme is responsible for degrading a particular type of fat known as cholesterol ester (esterified cholesterol combined with triglycerides) (Figure 1), and the deficiency of this enzyme leads to the progressive buildup of cholesterol esters in vital organs such as the liver, spleen, adrenal glands, hematopoietic system, intestines and blood vessel walls, providing some frequent signs and findings in this disease such as liver disease and dyslipidemia.[2]

The early manifestation of LAL deficiency, when manifested in the first months of life, was named Wolman's disease.[3] In these cases, it is a more aggressive form of this disease, in which patients develop relentless diarrhea, difficulty in weight and height gain, adrenal calcification, being rapidly fatal up to the 6th month of life.[3]

The form considered "late" LAL deficiency was called CESD (Cholesteryl Ester Storage Disease), presenting the first symptoms usually after the first 6 months of life until adulthood.[3] In this most common form of the disease, liver fibrosis, cirrhosis, liver failure, stroke at an early age, increased risk of cardiovascular diseases (due to premature atherosclerosis) are the most frequent clinical manifestations.[1]

Due to the great clinical variability in the presentation of LAL deficiency, diagnostic delay is common and many patients may be incorrectly diagnosed as having "idiopathic liver fibrosis/cirrhosis" or "cryptogenic non-alcoholic hepatic steatosis".[4]

Regarding treatment, until recently there were no specific therapies for LAL deficiency, some drugs to control dyslipidemia (such as statins) did not bring effective results[1]; however, through studies in animal models with administration of a recombinant form of the LAL enzyme (sebelipase), it was possible to carry out clinical trials in humans that culminated in a phase III clinical study in 2015, which results demonstrated a significant decrease in transaminases, liver fat, LDL, triglycerides, and an increase in HDL.[1] Subsequently, organs such as the FDA (Food and Drugs Administration) and the EMA (European Medicines Agency) approved enzyme replacement therapy (ERT) for LAL deficiency (administered intravenously every 15 days).[1]

As it is a relatively recent therapy, there is still no long-term follow-up of this treatment, which will be important to determine whether the progression of the disease to liver failure can be achieved in patients who started therapy before permanent liver damage takes place.[1] Thus, it is important that patients undergoing therapy with the recombinant enzyme undergo continuous clinical monitoring to concerning the effects and limitations of this therapy over a longer period of time (and in the clinical context of everyday life, outside of a controlled clinical trial).[1]

We report here the case of the first Brazilian patient diagnosed with lysosomal acid lipase deficiency using the enzymatic measurement technique on filter paper.

CASE REPORT

This is a male, 12-year-old patient, first and only child of young, non-consanguineous parents, referred for investigation of prolonged cholestasis with hepatomegaly.

His gestation had no complications. He was born by cesarean section, at term, W = 2940g, Length 46.5cm, head circumference 33cm, chest circumference 32cm, with APGAR 8/9. The patient developed prolonged neonatal jaundice (initially due to suspected ABO incompatibility), requiring an exchange transfusion. He was discharged on the fifth day of life.

He did not present significant neurodevelopmental changes, walking at around 1 year of age, making sentences

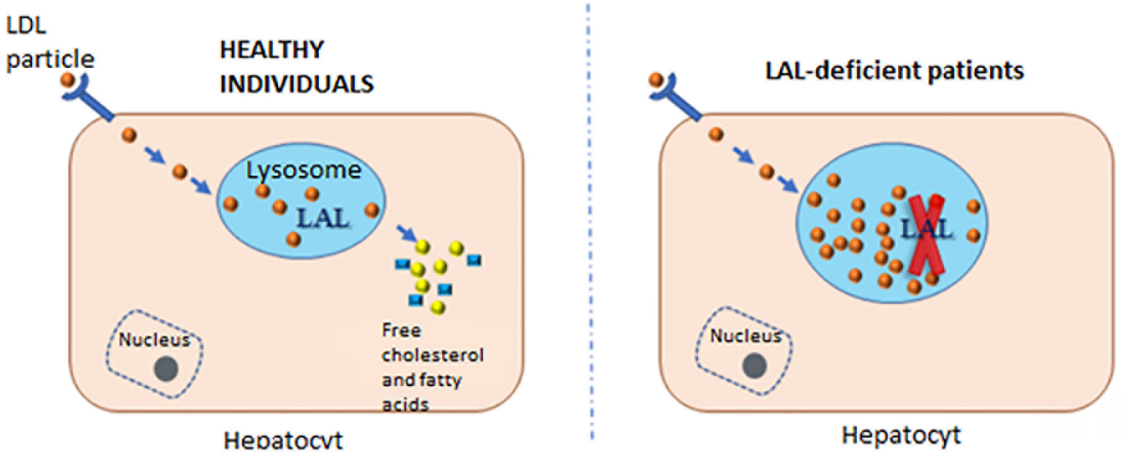


Figure 1. Schematic representation comparing a healthy cell with and cell with LAL deficiency. Legend: In lysosomal acid lipase (LAL) deficiency, there is an accumulation of cholesterol esters (orange dot) within the lysosome and a decrease in free cholesterol (yellow dot) in the cytoplasm) Source: The Authors (2021).

at 1 year and 6 months. Regarding his past medical history, the patient had dyslipidemia with a decrease in HDL, an increase in LDL, triglycerides and total cholesterol, in addition to a persistent increase in transaminases.

At the age of 3, he had enlarged spleen and liver, and he was referred for a liver biopsy, the result of which was compatible with a hereditary metabolic disease. Investigations were carried out for other metabolic diseases that involve liver disease, but the results were considered normal, with the exception of the filipin test for Niemann-Pick type C disease, which perinuclear fluorescence pattern was considered compatible with the diagnosis of this disease. Since this test may affect other diseases that interfere with the metabolism of intracellular cholesterol, diagnostic confirmation is necessarily carried out with molecular genetic analysis. Nonetheless, NPC1 and NPC2 gene sequencing was normal, ruling out this hypothesis.

Given the lack of diagnostic clarification, the patient underwent routine evaluation until an enzymatic test for lysosomal acid lipase deficiency was performed in Glasgow, Scotland, because, at the time, this enzymatic assay was not yet available in Brazil (Table 1). Enzymatic study on filter paper confirmed the diagnosis of lysosomal acid lipase (LAL) deficiency in the patient, later repeated in leukocytes and fibroblasts, corroborating results previously obtained on filter paper.

After the enzymatic diagnosis, more rigorous monitoring of dyslipidemia began. In their tests, there was a change in LDL and HDL cholesterol, high and low, respectively, and in the GOT and TGP liver enzymes, both increased (Table 2).

After the diagnosis, a low-fat and fiber-rich diet was introduced, aiming to control dyslipidemia, avoiding polyunsaturated fats, processed foods and excess carbohydrates.

His most recent clinical genetic examination revealed: height and weight between the 25th and 50th percentiles; head circumference between the 50th and 97th percentiles, without infiltrated facies, presence of hepatosplenomegaly, grade 2 osteotendinous reflexes in the lower and upper limbs, absence of signs of spinal cord compression and oculomotor apraxia, normal-size patient with diadochokinesis; preserved deep sensitivity.

DISCUSSION

The diagnosis of lysosomal acid lipase deficiency (LAL-D) is challenging, as it shares the same clinical and laboratory characteristics as other conditions, such as non-alcoholic steatohepatitis, familial combined hyperlipidemia, and other more common metabolic problems.[3] LAL-D presents on a clinical spectrum from childhood to adulthood (Table 3), similar to other lysosomal storage diseases.[3]

Those who manifested symptoms in the first year of life had what is historically known as Wolman's disease, which has a rapidly progressive presentation, being described for the first time in 1956; whereas those who developed clinical manifestations in childhood were described as having a cholesterol esters storage disease (CESD), or more modernly LAL-D.[5] In Wolman's disease, affected infants were diagnosed with severe malnutrition, adrenal calcifications, hepatosplenomegaly and death in the first months of life. In contrast, CESD involves a broader clinical spectrum with presentations that can appear from childhood to adulthood, sometimes presenting as cryptogenic cirrhosis.[5]

Patients with LAL-D can often be diagnosed late or even incorrectly. Since a specific treatment is now available for this disease, the diagnosis of LAL-D should appear in the differential diagnosis of infants with severe malabsorption syndrome or prolonged neonatal cholestasis of unknown cause.[5]

Furthermore, "idiopathic" liver fibrosis and cryptogenic cirrhosis in children or adults should trigger a higher level of suspicion for LAL-D screening. Therefore, diagnostic images, such as ultrasound and liver biopsy, are relevant for the diagnosis (although they may be nonspecific in many patients) and demonstrate changes in liver morphology, which may be suggestive of LAL-D, such as microvesicular steatosis with involvement of Kupffer cells, fibrosis and deposition of cholesterol ester crystals (when electron microscopy is performed to analyze biopsied liver tissue).

As seen in the findings of the present case, the disease manifests as an idiopathic microvesicular hepatic steatosis. Thus, after this analysis it is possible to make a presumptive diagnosis of LAL-D.[6]

Table 1. Diagnostic tests in the patient's workup.

Tests	Sample results	Reference values
Serum chitotriosidase	29,330 nmol/h/ml	8.8 - 132nmol/h/ml
Beta-galactosidase enzymatic assay in leukocytes	195 nm/h/mg prot	78 a 280nm/h/mg prot
Lysosomal acid lipase enzyme assay on filter paper	<0.02pmol/punch/hr	0.50 a 2.30pmol/punch/hr
Beta-glucosidase enzymatic assay in leukocytes	10.64 nm/h/mg prot	10 a 45nm/h/mg prot
Filipin test	Positive	Negative
NPC1 and NPC2 genes molecular study	No mutations	

Source: The authors (2021).

Table 2. Lipidogram and liver function tests.

BIOCHEMICAL TESTS	SAMPLE RESULTS	REFERENCE VALUES
Total cholesterol	236 mg/dL	< 170 mg/dL
HDL	26 g/dL	> 45 mg/dL
LDL	180 mg/dL	> 100 mg/dL
GOT/AST	87 U/L	15 a 45 U/L
TGP/ALT	133 U/L	10 a 65 U/L

Source: The authors (2021).

Table 3. Clinical spectrum of lysosomal acid lipase (LAL-D) deficiency.

ADULTS/CHILDREN	**INFANTS**
Variable progression	Rapidly progressive
Relatively common symptoms	Liver failure
High total cholesterol	Premature death
High LDL	Persistent vomiting
Low HDL	Hepatosplenomegaly
Liver disease that may progress to fibrosis and cirrhosis	Adrenal calcification

Source: The authors (2021).

The specific test for diagnosing LAL deficiency is based on enzymatic activity in peripheral blood leukocytes, skin fibroblast cultures and, more recently, on enzymatic analysis performed on a drop of dried blood collected on filter paper, enabling rapid recognition and the easily transportable and readily available material for testing. The enzymatic test on filter paper can be used for the selective screening of patients presenting, for example, with non-alcoholic hepatic steatosis (especially in non-obese patients) and hepatosplenomegaly without a defined etiology (Table 4).

Because of the hereditary nature of this disease, diagnosed patients and their families are entitled to genetic counseling. In these cases, prenatal diagnosis and knowledge on the LIPA mutation carrier greatly accelerates and simplifies diagnosis.

The patient in question was initially diagnosed as having Niemann-Pick disease type C (NPC) and, subsequently, with enzymatic measurement of lysosomal acid lipase on filter paper, the correct diagnosis of LAL-D was made (with confirmation also from analyzing enzymes in leukocytes and fibroblasts, after carrying out the test on filter paper). In view of this, it appears that the use of enzymatic analysis on filter paper as a form of screening, carries several advantages such as easy transport of samples, which can be sent via mail in a conventional envelope, without the need for refrigeration[7], easy storage of samples, with activity preserved for up to six months at room temperature and for two years at 4°C;[8–10] smaller reaction volume, saving reagents and sample quantity, making it cheaper than using leukocytes, plasma or fibroblasts.[11,12]

Table 4. Suggestive signs and symptoms for investigation of LAL-D.

SIGNS/SYMPTOMS
High ALT and AST without apparent cause
Having hepatomegaly and/or splenomegaly without a defined etiology
Progressive liver dysfunction, “cryptogenic” liver fibrosis and/or cirrhosis
Non-alcoholic liver steatosis
Dyslipidemia
• Triglycerides > 300mg/dL
• LDL ≥ 160mg/dL
• HDL ≤ 40mg/dL (males); ≤ 50mg/dL (females)

Source: Adapted[2]

Treatment consists of children and adults with LAL deficiency (CESD) taking cholesterol-lowering medications ("statins" and/or ezetimibe) to control the "bad" cholesterol (LDL) in the blood. Although these medications can reduce cholesterol, they have not been shown to interfere with the underlying disease or prevent serious liver complications. Furthermore, there is evidence that in some cases these drugs can mask the disease progression.[4]

Enzyme replacement therapy has been approved in the European Union and the United States since 2015 and has been used with great effectiveness. Therefore, early diagnosis of this disease is critical and extremely important in order to offer the best possible treatment for the patient. Although liver histopathological findings may suggest this disease or lead to other differential diagnoses, currently the measurement of LAL enzymatic activity has become a fundamental tool in the early diagnosis of this disease, avoiding erroneous diagnoses (as in the case of the patient in this report) and enabling the initiation of specific therapy should be carried out before there is irreversible damage to target organs.

REFERENCES

1. Balwani M, Breen C, Enns GM, Deegan PB, Honzík T, Jones S et al. Clinical effect and safety profile of recombinant human lysosomal acid lipase in patients with cholesteryl ester storage disease. Hepatology 2013;58(3):950-7.
2. Wierzbicka-Rucińska A, Jańczyk W, Ługowska A, Lebensztejn D, Socha P. Diagnostic and therapeutic management of children with lysosomal acid lipase deficiency (LAL-D). Review of the literature and own experience. Dev Period Med 2016;20(3):212-5.
3. Tommaso AMAD, Barra FF de C, Hessel G, Moreno CA, Giugliani R, Escanhoela CAF. Importance of liver biopsy in the diagnosis of lysosomal acid lipase deficiency: a case report. Rev Paul Pediatr 2018;36(1):113-6.
4. Bay L, Canero Velasco C, Ciocca M, Cotti A, Cuarterolo M, Fainboim A, et al. Liver disease and dyslipidemia as a manifestation of lysosomal acid lipase deficiency (LAL-D). Clinical and diagnostic aspects, and a new treatment. An update. Arch Argent Pediatr. 2017 Jun;115(3):287-93.
5. Kuloglu Z, Kansu A, Selbuz S, Kalaycı AG, Şahin G, Kirsaclioglu CT, et al. The frequency of lysosomal acid lipase deficiency in children with unexplained liver disease. J Pediatr Gastroenterol Nutr. 2019 Mar;68(3):371-6.
6. Botero V, Garcia VH, Gomez-Duarte C, Aristizabal AM, Arrunategui AM, Echeverri GJ, et al. Lysosomal acid lipase deficiency, a rare pathology: the first pediatric patient reported in Colombia. Am J Case Rep. 2018 Jun;19:669-72.
7. Rodrigues MDB, de Oliveira AC, Müller KB, Martins AM, D'Almeida V. Chitotriosidase determination in plasma and in dried blood spots: a comparison using two different substrates in a microplate assay. Clin Chim Acta. 2009;406(1-2):86-8.
8. Gasparotto N, Tomanin R, Frigo AC, Niizawa G, Pasquini E, Blanco M, et al. Rapid diagnostic testing procedures for lysosomal storage disorders: alpha-glucosidase and beta-galactosidase assays on dried blood spots. Clin Chim Acta. 2009 Apr;402(1-2):38-41.
9. de Castilhos CD, Mezzalira J, Goldim MP, Coelho JC. Influence of pre--analytical factors on α-galactosidase A, arylsulfatase B and α-glucosidase activities measured on dried blood spots on filter paper. Clin Biochem. 2011 Jul;44(10-11):922-6.
10. Castilhos CD, Mezzalira J, Goldim MPS, Werlang FG, Coelho JC. Effect of sample collection, temperature and time of storage on β-galactosidase and total hexosaminidase activities in dried blood collected on filter paper. Clin Chem Lab Med. 2011;49(8):1299-302.
11. Civallero G, Michelin K, de Mari J, Viapiana M, Burin M, Coelho JC, et al. Twelve different enzyme assays on dried-blood filter paper samples for detection of patients with selected inherited lysosomal storage diseases. Clin Chim Acta. 2006 Oct;372(1-2):98-102.
12. Benevides GN, Miura IK, Person NC, Pugliese RPS, Danesi VLB, Lima FR, et al. Lysosomal acid lipase deficiency in Brazilian children: a case series. J Pediatr (Rio J). 2019 Sep-Oct;95(5):552-8.