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DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF CHILDREN WITH LYSOSOMAL ACID LIPASE DEFICIENCY (LAL-D). REVIEW OF THE LITERATURE AND OWN EXPERIENCE

DIAGNOSTYKA I POSTĘPOWANIE TERAPEUTYCZNE U DZIECI Z NIEDOBOREM LIZOSOMALNEJ KWAŚNEJ LIPAZY (LAL-D). PRZEGLĄD LITERATURY I DOŚWIADCZENIA WŁASNE

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Abstract

Lysosomal acid lipase deficiency may present at any age (in infants, children and adults). Its presenting features commonly include elevated serum transaminase activity levels, hypercholesterolemia, fatty liver, progressive liver fibrosis, and cirrhosis. Nonspecific clinical manifestations can lead to a delay in the diagnosis of both children and adults. The early development of fibrosis and cirrhosis suggests that the lysosomal accumulation of cholesterol esters and triglycerides in the liver is a potent inducer of fibrosis. Elevated levels of low-density lipoprotein-cholesterol or low levels of high-density lipoprotein-cholesterol with elevated transaminase activity should raise the suspicion of lysosomal acid lipase deficiency in the diagnostic workup. Still, some patients may not present with abnormal triglyceride and cholesterol concentrations. Early onset LAL-D has a different clinical presentation, with acute symptoms, including liver failure, and can be confused with many other metabolic conditions or with lymphohistiocytosis. The dried blood spot test enables rapid diagnosis and should be widely applied when the cause of liver disease remains unknown.

Key words: lysosomal acid lipase deficiency (LAL-D), cholesteryl ester storage disease, LIPA deficiency, Wolman disease

Streszczenie

Niedobór lizosomalnej kwaśnej lipazy (ang. Lysosomal acid lipase deficiency, LAL-D), może pojawić się w każdym wieku (u niemowląt, dzieci i dorosłych). Objawem choroby często jest wzrost aktywności aminotransferaz, hipercholesterolemia, stłuszczenie wątroby, postępujące zwłóknienie wątroby, a także marskość. Niespecyficzne objawy kliniczne mogą doprowadzić do opóźnień w diagnostyce u dzieci i dorosłych. Enzym LAL uczestniczy w reakcji odłączania kwasów tłuszczowych od triglicerydów i estrów cholesterolu w obrębie lizosomu. Podwyższone stężenie LDL-C (lipoprotein o niskiej gęstości) lub niskie stężenie HDL-C (lipoprotein o wysokiej gęstości) powinny wzbudzić podejrzenia niedoboru lizosomalnej kwaśnej lipazy. Wczesny rozwój LAL-D jest niespecyficzny, może przebiegać z objawami takimi jak ostra

niewydolność wątroby i może być mylony z wieloma różnymi chorobami metabolicznymi lub zespołem hemofagocytarnym. Test suchej kropli krwi umożliwia szybkie rozpoznanie niedoboru kwaśnej lipazy i powinien być wykonywany w wybranych przypadkach chorób wątroby o nieustalonej etiologii.

Słowa kluczowe: niedobór lizosomalnej kwaśnej lipazy (LAL-D), choroba spichrzania estrów cholesterolu (cholesteryl ester storage disease, CESD), niedobór LIPA, Choroba Wolmana

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INTRODUCTION

Lysosomal acid lipase deficiency (LAL- D) (OMIMD 278000) is an autosomal recessive disorder caused by mutations in the *LIPA* gene resulting in enzyme deficiency and the accumulation of lysosomal cholesteryl esters (CEs) and triglycerides (TGs) [1]. Lysosomal acid lipase (LAL) is also called acid esterase and cholesterol ester hydrolase [EC 3.1.1.13]. The LAL enzyme participates in the reaction of fatty acid disconnection of triglycerides and cholesterol esters within the lysosome. Free fatty acids (FFA) after the transportation to the cytoplasm are bound by fatty acid binding proteins (FAB-fatty acid- binding proteins) and transferred to various cell organelles. Free cholesterol is taken up by a protein, NPC2, and as a complex binds to the protein NPC1 present in the lysosomal membrane. After leaving the lysosome, free cholesterol goes to the cell membrane or endoplasmic reticulum, where it inhibits the *de novo* synthesis of cholesterol and lipoprotein LDL, and also stimulates the synthesis of cholesterol esters [2]. The membrane anchored lysosomal hydrolases include some proteins and transporters responsible for the movement of various chemical compounds with lysozyme light into the cytosol and in the opposite direction. Disorders associated with deficient activity of lysosomal enzymes may occur as a congenital enzyme synthesis and folding, abnormal activation (e.g. Sapozyn absence) of the movement of enzymes within the cell, the post-translational processing of the enzyme molecules and the lack of functional lysosomal membrane proteins. LAL deficiency is characterized by hepatomegaly, increased transaminases, hypercholesterolemia, and the fatty liver disease. Because LAL D is an autosomal recessive disease, the absence of a family history of cardiovascular disease and/or hypercholesterolemia and current inconsistencies in pediatric lipid screening may delay early recognition of the lipid abnormalities in LAL D patients, in contrast to the other autosomal dominant inherited conditions, such as familial hypercholesterolemia, where family history can help in early diagnosis [3].

CLINICAL MANIFESTATION

Complete or partial inhibition of enzyme activity results in the accumulation of LAL unevenly distributed cholesterol esters and triglycerides in the cells and tissues of patients. Disorders of releasing free cholesterol from its esters lead to an increased synthesis of endogenous cholesterol and increasing LDL transport into the cell, and increased production of apolipoprotein B. The deficit of LAL activity is a rare autosomal recessive disease. LAL protein is encoded by the LIPA gene located on chromosome 10q23.2. The most common mutation is a deletion of the LIPA gene Δ254-2771 mutations in exon 8, which are responsible for the activity of LAL deficiency and lead to two phenotypes: Wolman disease and cholesteryl ester storage diseases (cholesteryl ester storage disease, CESD) [4].

Wolman disease is characterized by the complete or almost complete loss of activity LAL *in vivo*. Clinical symptoms appear in the first weeks of life of the patient and include an enlarged liver and spleen, vomiting, diarrhea, fat, stunting, psychomotor retardation, characteristic enlargement and calcification of the adrenal glands and fever. As a result of hepatosplenomegaly in children, increased abdominal circumference and breathing difficulties are observed. Wolman disease is severe and most patients die in early infancy [5]. Although it changes the metabolism of all the tissues of the patient's body, the accumulation of cholesterol esters, triglycerides and cholesterol is to the greatest extent observed in the liver and spleen.

In patients with cholesterol ester storage material (CESD) there is a residual LAL activity *in vivo* and it is associated with a more benign phenotype [6]. Abnormalities in lipid metabolism in the clinical picture appear in the first or second decade of life. The main clinical feature of the CESD is hepatosplenomegaly. Increase in total cholesterol and LDL lead to fatty liver. In addition, hypercholesterolemia is a cause of cardiovascular disorders. CESD should be suspected in the differential diagnosis of chronic liver disease of unknown etiology (atypical fatty liver without the presence of obesity), as well as in patients with dyslipidemia (hypercholesterolemia with reduced levels of lipoprotein HDL) [7]. In contrast to familial hypercholesterolemia (FH) inherited in the autosomal dominant way (heterozygote frequency of approx. 1:500), in patients with CESD, family history of hypercholesterolemia is negative (unless it states co-occurrence with FH). Physical examination, a detailed history of the family together with the results of basic laboratory tests assessing liver function are important diagnostic clues in children with hypercholesterolemia. The incidence of LAL deficit is not accurately estimated, but it is assumed to be less than 1 in 100 000 live births [8]. Such changes in LAL activity could contribute to the atherosclerotic process. The formation and accumulation of foam cells within wall arteries is a key pathophysiological moment in the formation of atherosclerotic plaque.

DIAGNOSIS

The basic test for the diagnosis of deficit LAL is based on enzyme activity in peripheral blood leukocytes, cultured skin fibroblasts, and more recently in dry blood spot (DBS),

which enables rapid recognition and easy, readily available transport material for testing. The test was performed as described previously by Hamilton *et al.* [9]. DBS values of 0.37-2.30 nmol/punch/h were interpreted as normal, 0.15-0.40 nmol/punch/h as carriers and <0.03 nmol/punch/h as CESD patients. DBS is used for the selective screening of patients with fatty liver, and hepatosplenomegaly without obesity. Because of the hereditary nature of the disease, probands and their families are entitled to genetic advice. In such cases, prenatal diagnosis and the determination of carrier knowledge of the mutations in LIPA greatly speed up and simplify the diagnostic process [10].

In lab tests anemia and thrombocytopenia can be seen. The serum concentration of cholesterol, LDL-chlesterol and triglycerides are increased. The disease may be accompanied by increased bilirubin, ALT and AST. The imaging of the abdominal cavity in patients with symptoms of lysosomal acid lipase deficit shows an enlarged liver, spleen and occasionally adrenal calcification. The histopathological examination of liver biopsies shows the accumulation of cholesterol esters. In a study using liver lipid chromatography, features of LAL deficits were also identified [11].

The long duration between initial disease symptoms and diagnosis in some patients is a significant problem but consistent with the situation involving other metabolic diseases [12]. Poor awareness and knowledge of LAL D is one of the reasons. In particular, we noted that abnormalities of hepatomegaly, elevated liver enzymes and/or fatty liver and dyslipidemia, may be confused with more common disorders, such as nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), metabolic syndrome, and/or familial hypercholesterolemia [13]. In contrast to other rare diseases characterized by early mortality and morbidity, disease progression in LAL D can occur relatively silently - therefore one potential explanation for the underrepresentation of older patients (though requiring further investigation) is that these patients die prematurely and that in some cases death may occur without the definitive diagnosis of LAL- D. Moreover, although persistent liver injury is present from early life, the serum transaminase levels alone may not lead to a specialist referral and/or a detailed liver workup [14]. The high LDL-C or low HDL-C levels seen in patients with LAL D, however, provide an opportunity to potentially distinguish these patients earlier during the diagnostic workup. LAL D seems to be a predominantly pediatric disease that primarily targets the liver, causing progressive hepatic dysfunction with fibrosis (and commonly cirrhosis), which, combined with the systemic manifestations of dyslipidemia, put these patients at the risk of significant morbidity and early mortality [15].

EXPERIENCE WITH SCREENING FOR LAL-D IN POLISH CHILDREN

We performed the dried blood spot test in selected children from Poland who presented with increased transaminase of unknown origin or severe multiorgan disease in infancy. We excluded major liver diseases prior to LAL-D testing in all of them: Wilson disease, alpha-1-antytrypsin deficiency, HBV, HCV, CMV infection. We tested a group of children with overweight/obesity who presented with a sustained increase of transaminase activity in spite of weight reduction but the majority of patients presented with normal weight. Among 89 children over 1 year of age we did not identify anyone with LAL-D. Still, one infant referred to our hospital with the suspicion of lymphohistocytosis was finally diagnosed to have early onset LAL-D. The DBS test turned out to be very easy to perform - samples were collected in out-patient clinics in Warsaw and Bialystok and transferred to the Psychiatry and Neurology Institute for biochemical testing. In one infant it was possible to make the diagnosis within 14 days after admission.

TREATMENT

LAL-D is usually a slowly progressing disease with cirrhosis and lipid disturbances which can not be completely cured by conventional therapy. Lipid-lowering medication (mainly statins) is commonly used in children with LAL-D. Statins can be introduced in children under 10 years of age in case of significant hypercholesterolemia which does not respond to dietary therapy. In general, patients with LAL-D require monitoring of their lipid parameters and their liver function test to make the right judgment on additional pharamacotherapies [16]. Patients who had undergone liver transplantation generally improved their clinical status, including lipid metabolism. Some of them had dyslipidemia and lower albumin levels before liver transplantation, which improved later on treatment [17]. Causal treatment was not available until recently, when sebelipase alfa treatment was approved. Sebelipase alfa is a recombinant human LAL enzyme (Alexion Pharmaceuticals, Inc.) which was tested in a randomized clinical trial and showed the sustained reduction in transaminases, improvements in serum lipid profile and reduction in hepatic fat content [18].

Table I. Who Should Be Tested for LAL Activity adopted from Baratta F et all [8].

Who Should Be Tested for LAL Activity:	Patient with higher elevation of ALT, AST
	Patient with liver dysfunction
	Patient with presence of hepatomegaly
	Patient with hepatic steatosis
	Patient with dyslipidemia: TCH >300 mg/dl-7,77 mmol/L High LDL-C ≥160 mg/dL–4.1 mmol/L Low HDL-C ≤40 mg/dL–1.0 mmol/L in males; ≤50 mg/dL–1.3 mmol/L in females

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