WOLMANO LIGA

WOLMAN DISEASE

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ABSTRACT

Key words: lysosomal acid lipase deficiency, Wolman disease, cholesterol ester storage disorder.

Wolman disease is an autosomal recessive storage disorder caused by a deficient activity of lysosomal acid lipase (LAL). The disease leads to massive accumulation of triglycerides and cholesterol esters in most tissues of the body and is nearly always fatal in infancy. The abnormality of lipid metabolism becomes clinically evident in the first weeks of life. Progressive hepatosplenomegaly, vomiting, steatorrhea, failure to thrive and adrenal calcification are major clinical findings. The diagnosis of Wolman disease requires clinical experience and specialized laboratory tests. The diagnosis is based on finding absent activity of acid lipase and/or molecular tests. Early diagnosis is particularly important for bone marrow transplantation and in the near future enzyme replacement therapy. Human trials with recombinant LAL are currently underway, raising the prospect for specific correction of LAL deficiency in Wolman disease.

Cholesterol ester storage disease is caused by a partially deficient activity of lysosomal acid lipase and has a more variable clinical course.

The purpose of this review is to present diagnostic difficulties associated with clinical picture of Wolman disease, biochemical and genetic methods used to confirm the diagnosis and therapeutic possibilities.

SANTRAUKA

Reikšminiai žodžiai: rūgštinės lizosomų lipazės trūkumas, Wolmano liga, cholesterolio esterių kaupimasis.

Wolmano liga yra paveldima autosominiu-recesyviniu būdu kaupimo liga, kuri išsivysto dėl rūgštinės lizosomų lipazės trūkumo. Šis trūkumas sukelia trigliceridų ir cholesterolio esterių kaupimą, tai dažniausiai miršta dar kūdikystėje. Liga diagnozuojama remiantis klinikiniais simptomais ir specifiniais tyrimais. Pagrindiniai klinikiniai požymiai yra progresuojanti hepatosplenomegalija, vėmimas, steatorėja, nepakankamas svorio priaugimas, sulėtėjęs vystymasis ir antinės kalcifikacijos. Specifiniais ir molekuliniais tyrimais nustatomas rūgščios lipazės trūkumas leukocitose, odos fibroblastuse ar sauso kraujo lašė. Jie pavyksta anksčiai nustatyti diagnozę, atliekama kaulų kūno transplantacija, vėliau skiriama pakaitinė fermento terapija. Šiuo metu atliekami tyrimai, kuriais bandoma panaudoti rekombinantinę rūgštinę lipazę.

Šio darbo tikslas yra apžvelgti diagnostikos sunkumus, susijusius su Wolmano liga klinikai, biocheminius ir genetinius tyrimų metodus, kurie patvirtina diagnozę ir gydymo galimybės.

DEFINITION, HISTORIC BACKGROUND AND INCIDENCE

Lysosomal storage disorders (LSD) constitute a group of more than 40 different, genetically conditioned diseases resulting from specific deficiencies in lysosomal functions.

In the case of Wolman disease (OMIM 278000), the deficient enzyme lysosomal acid lipase (acid esterase, cholesterol ester hydrolase; EC 3.1.1.13), is responsible for the hydrolysis of cholesterol esters and triglycerides at low pH inside lysosomes. The disease is inherited in an autosomal recessive pattern.

The first description of an infant with abdominal distension, massive hepatosplenomegaly and calcification of the adre-
nal glands was published in 1956 by Wolman [1] who in 1961 reported other siblings with similar symptoms from the same family [2]. Initially he named the disease “generalised xanthomatosis with calcified adrenals” [1, 2]. Crocker et al. suggested an eponymic name of Wolman disease [3]. Since that time the literature has presented only few cases of subsequent patients.

Cholesteryl ester storage disorder (CESD, OMIM 278000) has a more variable clinical course and but is also caused by deficient activity of lysosomal acid lipase.

The incidence of Wolman disease is not known precisely; however, it is estimated to be less than 1 per 100 000 live births [4, 5]. The results of screening tests in a group of Iranian Jews in Los Angeles suggest that 1 per 4200 newborns in this population can be affected by Wolman disease. However, the fact that it is an insulated genetic population, not representative e.g. for European population, has to be taken into account [6]. A great number of lysosomal diseases, similarly to other autosomal recessive diseases, develop significantly more often in isolated populations what is a result of a founder effect and marriages between closely related persons; it may be conditioned by a geographical, religious or social situation.

AETIOLOGY AND PATHOGENESIS

Wolman disease is caused by a complete deficiency of lysosomal acid lipase (LAL). Cholesterol esters and triglycerides reach the inside of the cells using LDL, through endocytosis with LDL receptors. Endocytic vesicles can fuse with the compartment of early and late lysosomes. As a result of LAL activity cholesterol esters and triglycerides are cleaved and free fatty acids are generated, and they are directed to mitochondria, peroxysomes and endoplasmic reticulum. Some free cholesterol reserves are directed to the cytoplasmic membrane and some to the endoplasmic reticulum where they inhibit the synthesis of free cholesterol.

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**Clinical symptoms:**
- hepatomegaly
- abdominal distension/gastrointestinal symptoms (vomiting/diarrhoea/flatulence)
- failure to thrive

**Other clinical symptoms:**
- rapidly progressing cachexia
- jaundice
- splenomegaly
- elevated body temperature

**Laboratory test abnormalities:**
- high normal/elevated levels of Chol, LDL, TG
- elevated levels of transaminases, bilirubin
- anaemia, thrombocytopenia
- X-ray: enlarged adrenals with calcifications

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**Wolman disease is suspected**

- Enzymatic tests – lysosomal acid lipase deficiency in isolated leukocytes or cultured skin fibroblasts or dry blood spot testing
  Thin layer chromatography of liver lipids
- Genetic analysis

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**The time of making the diagnosis and starting enzymatic treatment are crucial**
de novo. In the case when LAL enzymatic activity is diminished, free fatty acids and free cholesterol are not released. Cholesterol esters and triglycerides are stored; as a result the synthesis of free cholesterol de novo is stimulated.

CLINICAL CHARACTERISTICS

The majority of patients with Wolman disease have a very similar clinical manifestation. The disease usually develops within the first weeks of life with symptoms of increased vomiting and diarrhoea, hepatosplenomegaly and rapidly progressive cachexia. Cholestasis and elevated body temperature is observed almost always [1, 3]. Death usually occurs between 3 and 6 months of life [7].

Anaemia usually develops at around 6th week and progresses along with the disease. The results of other haematological tests indicate lymphocyte vacuolization and an increased number of foam cells in the bone marrow that at later stages of the disease are also present in the peripheral blood. The cholesterol and triglyceride blood levels are usually normal [7].

Hepatosplenomegaly is a constant feature of this disease and can be extensive, leading to breathing limitations due to mechanical chest compression. It has been reported even as early as on the 4th day of life.

A symptom which draws the most attention, though not always present, but is pathognomonic for Wolman disease is calcified and symmetrically enlarged adrenal glands. Enlarged liver and calcified adrenals are visible on plain X-ray images of the abdominal cavity, and on ultrasound and CT scans as well.

Symptoms related to the central nervous system are rare; however, the psychomotor development is delayed.

DIAGNOSTICS

Wolman disease is suspected based on a clinical picture (Table 1, Figure 1).

<table>
<thead>
<tr>
<th>Test type</th>
<th>Material type</th>
<th>Test advantages</th>
<th>Test limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes</td>
<td>Heparinised whole blood EDTA whole blood</td>
<td>Concentrated homogenous samples. Numerous enzymes can be analysed in one sample.</td>
<td>Difficulties preparing and sending material.</td>
</tr>
<tr>
<td>Cultured fibroblasts</td>
<td>Punch skin biopsy</td>
<td>One type of cells. Numerous enzymes can be analysed in one sample.</td>
<td>An invasive procedure. Preparing and sending the material. Long (weeks) time for sample growth, long time waiting for the results.</td>
</tr>
<tr>
<td>Dry blood spot testing</td>
<td>Whole blood Heparinised whole blood applied on filter paper</td>
<td>Easy sampling and transport. Numerous enzymes can be analysed in one sample.</td>
<td>This test is not available in all parts of the world. It is considered to be a screening test (its result should be confirmed by enzyme testing in leukocytes or DNA analysis).</td>
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DNA analysis

Sequencing the whole LIPA gene | High detectability in patients. It can confirm the results of enzyme activity tests. | Potentially more expensive. Pathogenic properties of unclassified or “private” mutations have to be confirmed. |
tests, most frequently para-nitrophenol or 4-methylumbelliferone.

MICROSCOPIC EXAMINATIONS
Under a light microscope, the presence of foam cells (lipid-loaded macrophages) in a bone marrow smear or vacuolated lymphocytes in a blood smear may be present in patients afflicted with Wolman disease. Using an electron microscope, typical free cholesterol needle-like crystals and so-called lipid droplets can also be observed in the hepatocytes for Wolman.

Other biochemistry tests
Until recently, thin layer chromatography of lipids isolated from a liver biopsy specimen was required to diagnose LAL deficiency. Using this test it is possible to observe a profile of lipid storage with massive amounts of cholesterol esters, triglycerides and free cholesterol which is typical of Wolman disease/CESD. Due to its invasiveness, a liver biopsy is performed very rarely.

Molecular tests
Wolman disease is related to different mutations of the LIPA gene located on the 10q23.2–q23.3 chromosome coding a protein of lysosomal acid lipase.

In the event of Wolman disease gene rearrangements, including deletions, insertions or nonsense mutations, lead to the lack of or extremely low activity of acid lipase. LIPA gene mutations leading mainly to the inhibition of protein synthesis, exon deletion, defective splicing of transcripts are causes of the lack of or very low LAL activity.

Genetic counselling
Genetic counselling is indicated for parents and siblings of probants in order to determine a risk of child being sick. If a mutation(s) in the family is known the diagnosis can be rapidly achieved using a molecular analysis of collected material. Currently screening tests for newborns are not available.

THERAPEUTIC MANAGEMENT
So far only palliative treatment was available and included blood transfusion to alleviate anaemia and compensation of adrenal insufficiency.

BONE MARROW STEM CELL TRANSPLANTATION
The literature reports several descriptions of patients with Wolman disease treated with bone marrow transplant. Four of them died due to complications associated with the procedure, and improvement was observed in one patient [9].

Enzyme replacement therapy (ERT)
Studies with recombinant LAL in patients with Wolman disease are currently under way (www.clinicaltrials.gov) identifier: NCT01371825 [10].

CONCLUSIONS
Wolman disease is a rare lysosomal storage disease inherited in an autosomal recessive pattern.

Disease symptoms develop during infancy and almost always lead to death until the age of one year. Hepatosplenomegaly, fatty stools, increased waist circumference, other gastrointestinal symptoms, adrenal calcifications visible on X-ray images and inhibited development are observed as early as from the first weeks of life.

An initial diagnosis is based on a clinical manifestation, and in order to diagnose this disease it is necessary to confirm lack of lysosomal acid lipase activity and/or molecular tests.

Studies with recombinant lysosomal acid lipase in patients with Wolman disease are currently under way, giving a chance for treatment of this disease.

REFERENCES