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SPECIFIC ENZYMATIC DIAGNOSIS AND ULTRASTRUCTURAL ANALYSIS OF PERIPHERAL LEUKOCYTES IN INFANTILE FORM OF NIEMANN-PICK SPHINGOMYELINOSIS

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Acute infantile type of Niemann-Pick sphingomyelin lipidosis has been published concentrating to the morphological and biochemical specific enzyme investigations.
The electronmicroscopical study of the peripheral lymphocytes proved to be of diagnostic value for lysosomal lipidoses.

INTRODUCTION

Sphingomyelinase deficiency as a specific biochemical defect in Niemann-Pick disease (NPd) has been proved by Brady et al /1/. Further step has been the detection of the spingomyelinase A and B isozymes /2/. Use of artificial substrates has further facilitated the diagnosis of the different genotypes and the prenatal diagnosis of this autosomal-recessive disease /8, 6, 10/.

There are three or four types of the NPd, so the acute infantile form /type A in Crocker's classification/; chronic visceral form /type B in Crocker's classification/ and the juvenile or subacute form of NPd (type C.). The type D. the Nova Scotian variant has been described by Crocker and Farber /4/. Fredricksen and Sloan /7/, Schneider and Kennedy /12/.

In our girl infant patient suffering from NPd specific enzymatical and morphological investigations were performed.

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CASE REPORT

Eva F. (born 5.Sept. 1983) developed psychomotorically normal until her 7 months of age. Her skin lesions were suspected for histiocytosis X. Neurological and ophthalmological examinations were normal, enlarged abdomen, hepatosplenomegaly was only detected (8 cm both of them).

Bone marrow aspiration biopsy showed no lipid accumulating cells or any signs of malignant haematological disease. In the liver biopsy (No 5718/1984) material there were seen mononuclear cells with foamy cytoplasma and intralobular and portal fibrosis. According to the histological picture of the second liver biopsy (4.Dez.1984) NPd was suspected, "foam cells" were present containing lipid droplets in the cytoplasm. The so-called " cherry red" macula appeared at the age of 12 months.

General muscle hypotonia, weak tendom reflexes, retarded mental development were seen after 1 year of age.

After recurrent respiratory infections the patient died at the age of 2.5 years.

The autopsic findings were in accordance with clinical diagnosis, foam cells were present in bone marrow, in the liver, in the spleen and other lymphatic tissues. The cerebral cortex was atrophized.

METHOD AND RESULTS

The electronmicroscopical examination of the peripheral lymphocytes was highly informative for the accumulation of lipid like material, the numerous intracytoplastic lipid droplets were membrane-limited and contained osmiophil materials (Figs. 1-2). The values of the lysosomal hydrolases (fucosidase, mannosidase, beta-galactosidase, N-acetylglucosaminidase) from the leukocyte homogenisate are seen in Table I. The enzyme activities are given in 1 mg protein content/l /8a/.

GM_1 gangliosidosis, Tay-Sachs disease , Sandhoff disease and GM_2 gangliosidosis had been excluded.

For the determination of the sphingomyelinase activity of the leukocytes as substrate the synthetic 2N (hexodecanoyl) amino-4-nitrophenyl phosphoryl-choline was used /1,8/. In the leukocytes a low activity was proven, with residual activity and in the liver homogenisate there was a very low specific enzyme capacity according to the diagnosis of NP sphingomyeline

TABLE I

Enzyme values from members of family F. with sphingomyelinosis

Leukocyte homogenisate	Éva F. µmol/l mg protein/l h	Controls n = 10
Fucosidase	126.3	111.8
Mannosidase	72.9	63.2
Beta-galactosidas	34.6	15.6
N-acetyl-beta-glucosaminidase A	2360.0	2108.0
" B	567.0	673.0

Sphingomyelinase µmol/g/min leukocyte	É. F.	mother	father	controls
	30	65	82	55-190
Liver homogenisate	16.8	-	-	

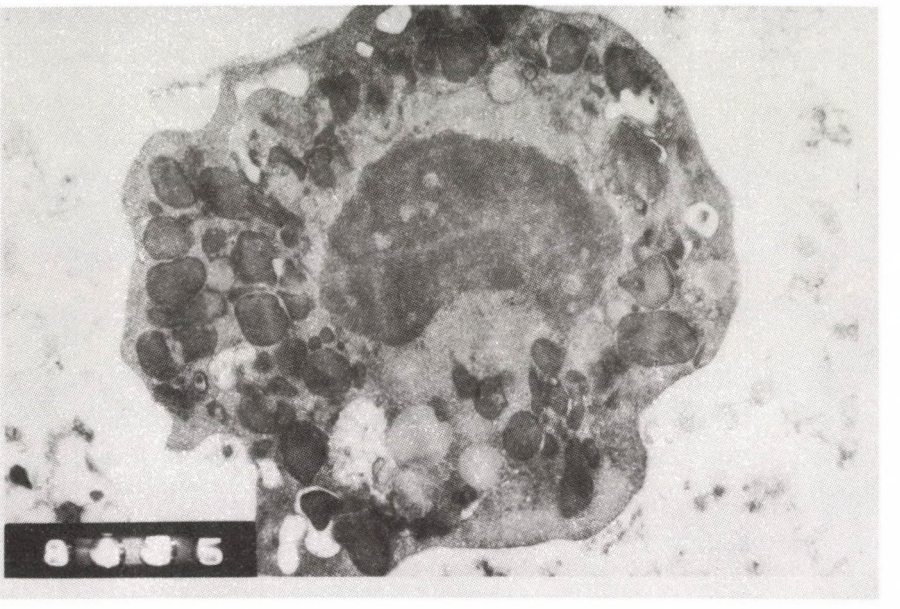

Fig. 1. Tem (transmission electron microscopy) micrograph of peripheral lymphocyte with intracytoplasmatic membrane limited osmiophil materials, x 7.800

lipidosis (Table I). According to the sphingomyelinase value the parents proved to be heterozygotes and the inheritance autosomal recessive.

X-rays revealed a very fine symmetric reticular density of the lungs and signs of the interstitial fibrosis.

She has got several times cell rich plasma infusions and different antibiotics for the intercurrent infections. At the age of 2 years 5 months she died of cardiorespiratoric insufficiency with extreme degree of hepatosplenomegaly.

DISCUSSION

The patients suffering from the most common type, the acute infantile form of NP-disease show failure to thrive, hepatosplenomegaly, cherry red macula in the early infant period. General muscle hypotonia, weak tendon reflexes, progressive

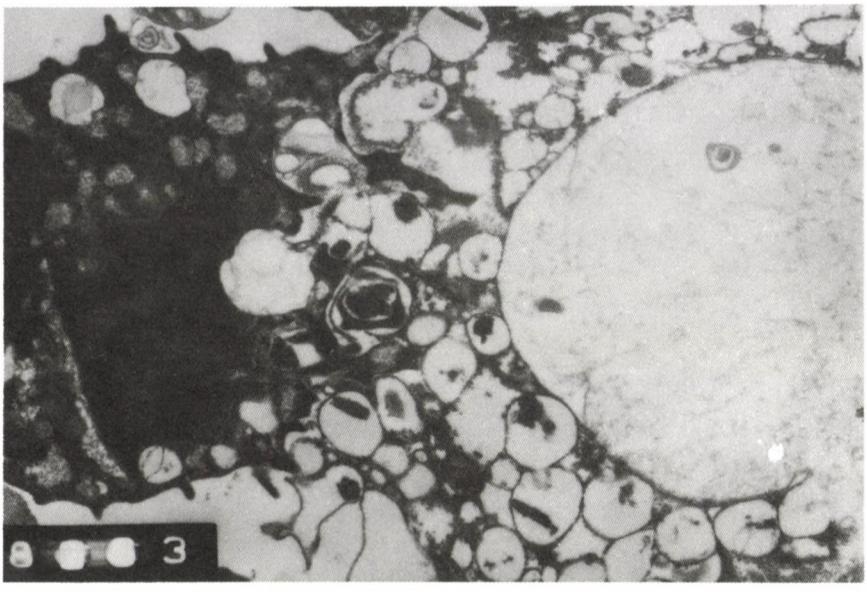

Fig. 2. TEM of lymphocyte and of a histiocyte, the cytoplasmatic vacuoles containing inclusions with semicurvilinear structure, x 6.240

deterioriation and recurrent respiratory infections and "foamy cells" in bone marrow are characteristic. Exitus lethalis may be expected at 1-4 years of age.

All of the above mentioned typical symptoms were seen at our infant girl patient.

The "foam cells" contain numerous membrane – bound cytoplasmatic bodies with myelin figures consisting of osmiophilic concentric structures /13, 3/.

According to our electronmicroscopical study of the peripheral leukocytes and lymphocytes intracytoplasmatic osmiophil lipid - like material was detected with different density without any myelin-structure.

Da Silva et al /5/ described cytoplasmatic inclusion bodies in peripheral nerves and demyelinisation.

The lipid accumulating foam cells can be observed in different tissues and organs, in the corneal tissue and in the epithelial cells and in the retina /11/. Conjunctival biopsy

has been recommended as diagnostic aid for histological and electronmicroscopic investigation by Libert and Dancis /9/.

Our method for the electronmicroscopical investigation of the peripheral lymphocytes must be easier and more available for diagnosis of the different types of lipidoses.

Enzyme diagnosis

The specific enzymatic defect was determined by Brady et al /1/ and the use of arteficial substrate was firstly described by Gal et al /8/.

The different types of NPd may be differentiated by the assay of the sphingomyelinase isozymes /2,3/.

For the detection of the gene-carriers for NPd leukocytes and cultured fibroblast cells can be used, although there may overlap with natural and with arteficial substrates /14/.

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