Case 13

GM2- Gangliosidosis in a cat

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HEMATOLOGY AND CHEMISTRY:

Biochemical abnormalities included a mild increase in phosphate (6,3 mg/dL, RI: 3,3-5,8 mg/dL) and CK (364 mg/dL, RI: 92-357 mg/dL) and a mild decrease in cholesterol concentration (55 mg/dL, RI: 68-224 mg/dL).
Puppies and kitten usually have a higher value of phosphate and CK than adult animals. The mild decrease in cholesterol concentration is possibly due to decreased synthesis due to decreased functional hepatic mass. Measure bile acids could be useful to better elucidate the pathogenesis of hypocholesterolemia. The mild normocytic normochromic non regenerative anemia is likely due to anemia of chronic disease and the moderate leukocytosis with lymphocytosis might be due to chronic antigenic stimulation. On blood smear examination the erythrocyte density appeared slightly decreased. Platelets were adequate in number, some giant platelets and platelet clumps were noted. The majority of lymphocytes contained multiple clear, variably-sized cytoplasmic clear vacuoles. A few clear vacuoles within the cytoplasm of monocytes were occasionally seen.
The vacuoles present within the lymphocytes and the monocytes are suggestive of a lysosomal storage disease. The lysosomal storage diseases described in cats and commonly associated with vacuolated lymphocytes includes: Fucosidosis, Mannosidosis, GM1-gangliosidosis and GM2-gangliosidosis. Fucosidosis is a deficiency of lysosomal α-fucosidases that implies the inability to breakdown fucose-containing compounds, resulting in their accumulation in various tissues in the body. Fucosidosis results in progressive neurological deterioration, skin abnormalities, growth retardation, skeletal disease and coarsening of facial features. Mannosidosis is a deficiency of β-mannosidase that leads to accumulation of disaccharides and trisaccharides with a terminal mannose β-linked to N-acetylglucosamine. Progressively worsening neurological signs that develop in affected cats include tremors, loss of balance, and nystagmus. Finally the gangliosidoses arise to the subgroup of lysosomal storage disorders called sphingolipidosis. Gangliosides are normal components of cell membranes, particularly neurons. There are two types of Gangliosidosis based on which ganglioside, GM1 or GM2 accumulates in lysosomes in the central nervous system and other body tissues.

FOLLOW UP:

We already knew that Zinnia had GM2 gangliosidosis because she belonged to one of the two colonies of cats with GM1 and GM2 gangliosidosis created by Dr. Baker in the Scott-Richies Research Center of Auburn University as an animal model to study these Lysosomal storage disordes. Dr. Baker and Dr. Martin have been testing Gene Therapy in cats with GM1 and GM2 gangliosidosis and the good results that they obtained strongly support the continued development of Adeno-associated viruses (AAV) vectors for human gangliosidosis clinical trials. The Gene therapy consists of the use of a Viral Vector to transfer a copy of the normal enzyme's cDNA to a patient's own cells. Adeno-associated viruses, from the parvovirus family, are small viruses with a genome of single stranded DNA. These viruses can insert genetic material at a specific site on the chromosome. Intracerebral gene therapy represents a promising approach for the treatment of CNS disease as it has the potential to provide a permanent source of the deficient enzyme on the parenchymal side of the blood brain barrier. Zinnia was humanely euthanized at the age of 4 months and a necropsy was performed.

HISTOPATHOLOGY:

Gross lesions were not found at necropsy. Histopathological examination of cerebral cortex section stained with hematoxylin-eosin revealed neuronal degeneration characterized by varying degree of swelling, cytoplasmic vacuolation, loss of Nissl substance, margination of nuclei (Fig. 1). The liver was diffusively affected. Hepatocytes and Kupffer cells were vacuolated and contained large amount of non staining material that often displaced the nucleus (hepatocellular lysosomal hypertrophy) (Fig. 2). Lymph nodes were diffusely infiltrated by macrophages with foamy vacuolated cytoplasm (Fig. 3). Abundant accumulations of vacuolated macrophages were observed also in the thymus (Fig. 4).
In frozen sections the cytoplasm of affected cells stains intensely with periodic acid-Schiff (PAS) and faintly with stain for neutral fats. The presence of ganglioside storage in neurons and glia can be confirmed by lectin histochemistry, (only on frozen section, because during the deparaffination of paraffin sections with xilene, ganglioside is extracted and therefore cannot be identified).

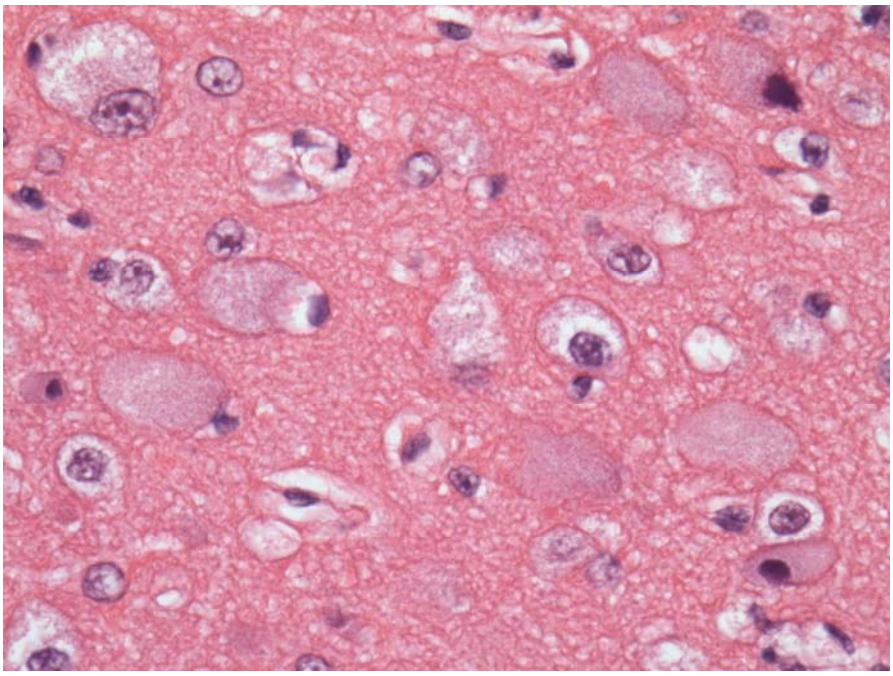

Fig. 1: cerebral cortex, hematoxylin-eosin, 40X

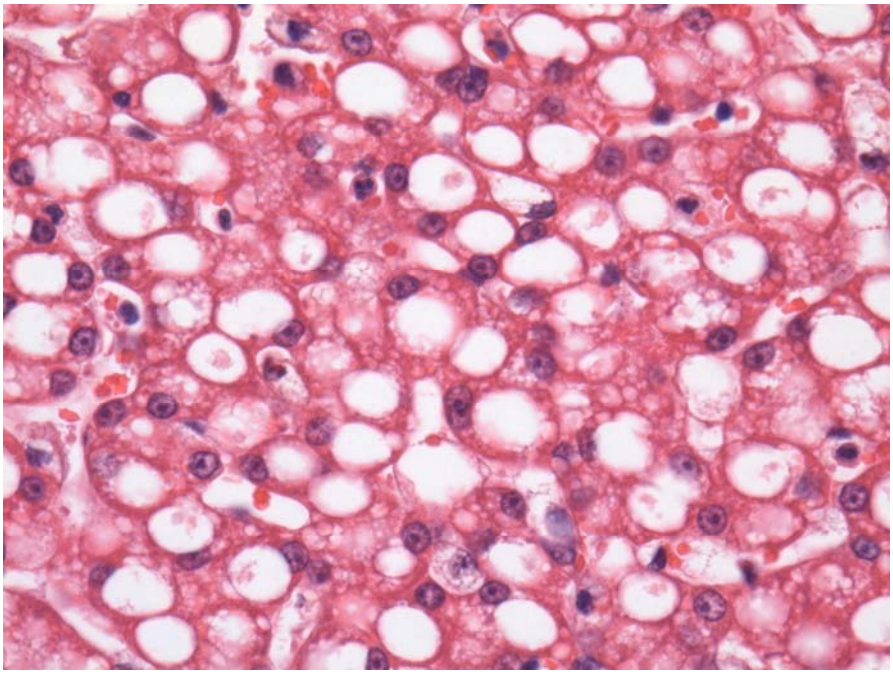

Fig. 2: Liver, hematoxylin-eosin, 40X

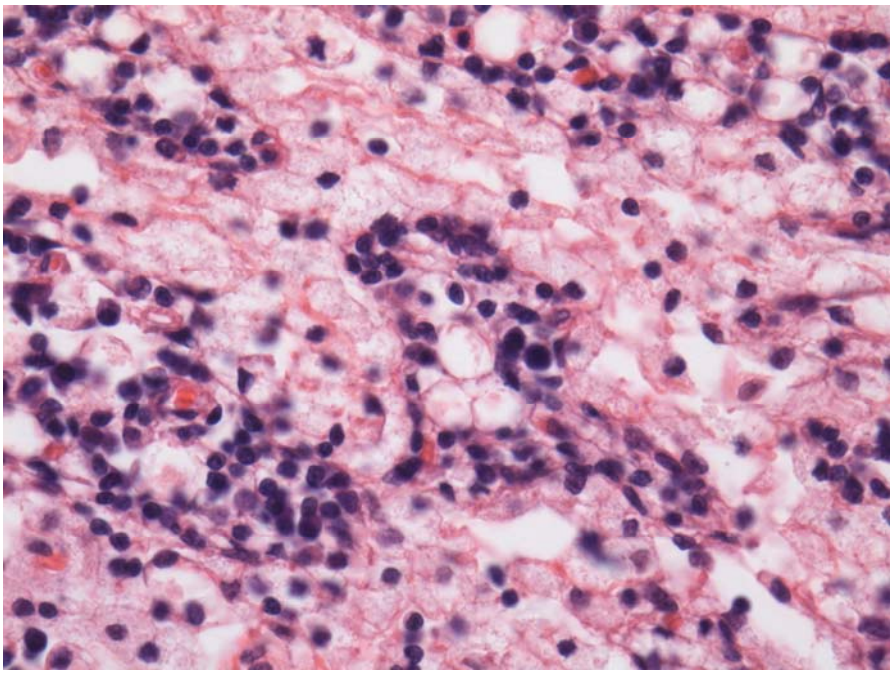

Fig. 3: Lymph node, hematoxylin-eosin, 40X

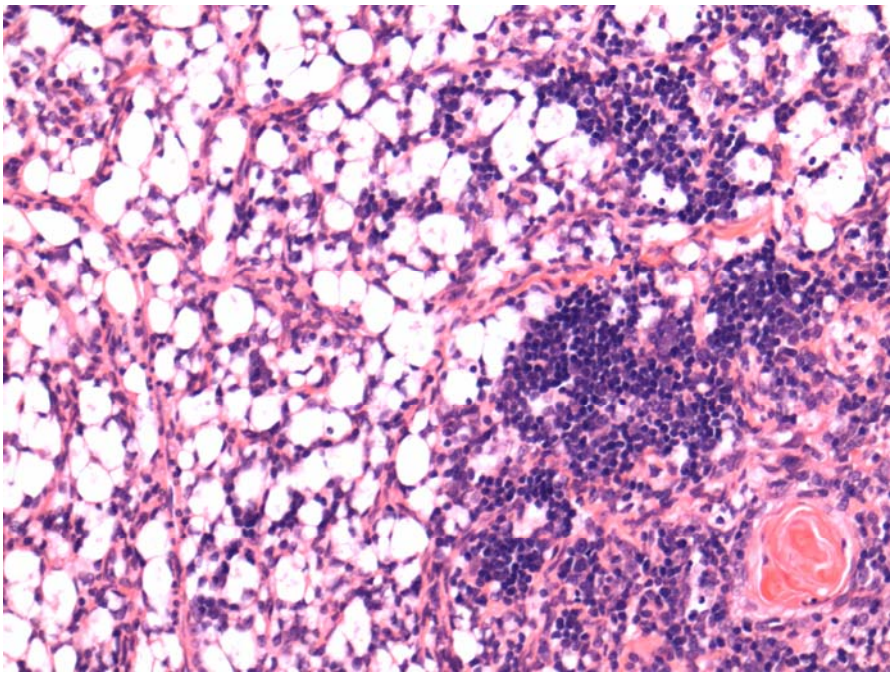

Fig. 4: Thymus, hematoxylin-eosin, 20X

DISCUSSION:

Lysosomal storage disorders (LSDs) arise from functional defects in one or more of the proteins essential to normal lysosome function. These defects typically involve the enzymes that play a critical role in the intracellular digestion of glycoproteins, glycolipids, glycosaminoglycans, or other macromolecules. The defect can involve: lysosomal enzymes, activator proteins that promote enzyme activity or transport proteins that move macromolecules into and out of the lysosome. GM2 Gangliosidosis has been described in korat, Burmese and DSH cats, Shorthaired pointer and Japanese Spaniel Dogs and people. The hydrolysis of GM2 ganglioside is catalyzed by lysosomal acid β-hexosaminidase. Functional hexosaminidase activity requires the coordinate action of three distinct proteins: one non hydrolytic GM2 activator protein and two hydrolytic subunits (α and β). Subunits may combine to form the two major hexosaminidase isoenzymes, each with two different substrates specificities: Hex B (ββ) and HexA (αβ). Only Hex A can act on the complex of GM2 ganglioside and GM2 activator protein. Defects in any of the three related genes may lead to GM2 gangliosidosis: HEXA, which encodes the α-subunit of Hex A; HEXB, which encodes the β-subunit of Hex A and Hex B; or GM2A, which encodes the GM2 activator protein. With a defect in the α or β subunit, beta-hexosaminidase A is unable to bind to GM2 activator protein and thus is unable to degrade GM2 ganglioside to GM3 ganglioside. Because GM2 ganglioside cannot be broken down, it accumulates in the lysosomes. These abnormal lysosomes filled with GM2 ganglioside accumulate in the cell and can block other cellular processes. All these deficiencies result in secondary accumulation of GM2 ganglioside and other complex oligosaccarides expecially in neuron and glial cells.

In patients with GM2 gangliosidosis the formation of spiny and aspiny enlargements at the axon hillock region (meganeurites) and the growth of secondary neurites from cerebral cortex (ectopic spines and neurites) are very often observed, especially in GABAergic (inhibitory) neurons (neuroaxonal dystrophy, Fig. 5). Electron microscopy images of neurons show multilamellar, spherical cytoplasmic inclusions, about 1 micrometer in diameter consisting of multiple concentric lamellae called membranous cytoplasmic bodies (Fig 6).

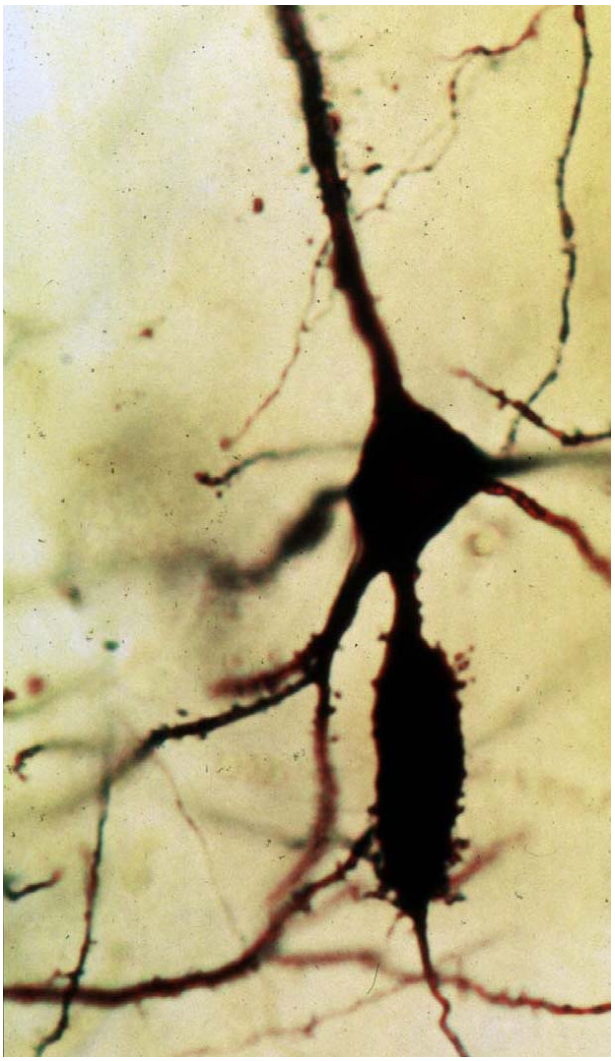

Fig. 5

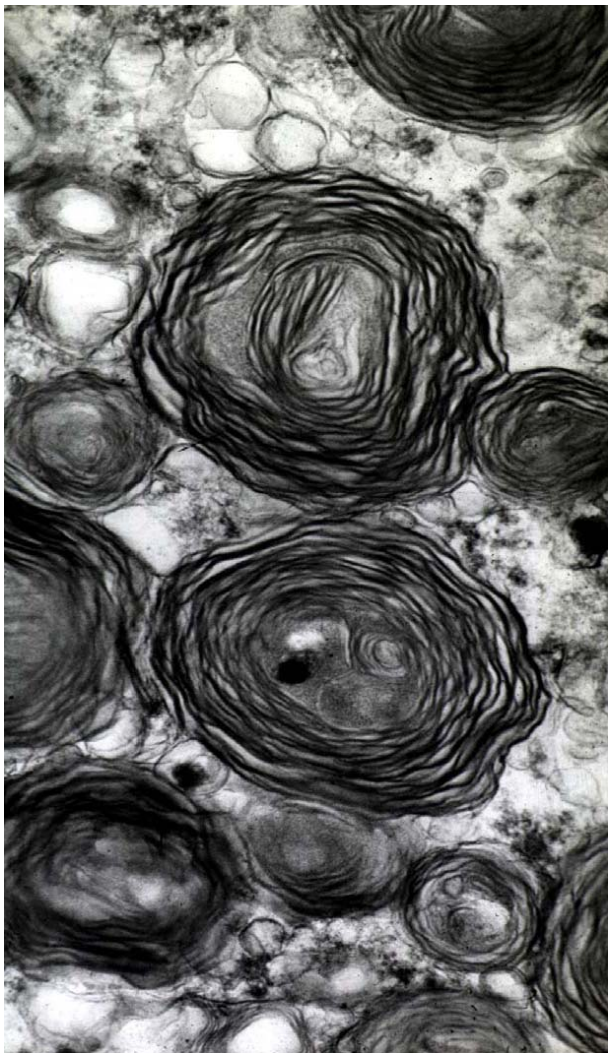

Fig. 6

Different possible explanations for the pathogenesis of neurological signs occurring in the gangliosidoses have been proposed, none of which are mutually exclusive. The abnormal accumulation of gangliosides and their metabolites could directly cause neurotoxicity (Suzuki, 1998), otherwise, meganeurites and ectopic dendrites characteristic of the gangliosidoses could lead to the onset and progression of neuronal dysfunction by altering electrical properties of the neurone (Walkley, 1998). The inappropriate apoptosis of neurones caused by accumulation of gangliosides could either directly or indirectly trigger the pathways of programmed cell death (Huang et al., 1997; Walkley, 1998). Another factor that has only recently been investigated in the genesis of neuronal dysfunction is inflammation (Wada, 2000). Chronic microglial activation and macrophage infiltration are prominent features in the CNS of patients and animal models with the gangliosidoses and other non-lysosomal storage disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Huntington's disease and prion diseases. The storage disorder activates resident microglial cells and recruites macrophages that cross the blood–brain barrier, generate oxygen-free

radicals, nitric oxide and other potentially toxic products, such as cytokines. The neurotoxicity and the accompanying oxidative stress are thought to contribute to the disease process.
In the live animal when a multifocal neurological disease has been recognized, lysosomal storage diseases should be among the differential diagnoses.
If a lysosomal storage disease is strongly suspected, two main routes can be taken to pursue a specific diagnosis in the live animal. The first relies on the identification of the deficient enzyme by assaying for the activities of a selection of lysosomal enzymes, including those known to have been reported as deficient in the breed of animal under investigation. This method provides a definitive diagnosis for the majority of lysosomal storage diseases. Ideally, an age-matched control should be assayed in parallel to provide a normal set of control values. For autosomal recessive diseases, an affected homozygote would be expected to have severely depleted enzyme activity (typically 0–5% of normal), whereas heterozygotes should have approximately 50% of the normal activity. Suitable substrates for antemortem enzyme analysis include whole-blood leukocytes, liver and kidney biopsy samples, and cultured skin fibroblasts.
The second specific diagnostic method thus relies on the identification of the genetic defect. Molecular genetic tests are available for feline GM1 and GM2 gangliosidosis and canine fucosidosis.
New forms of the same disease may be recognized, however, and the experience in human medicine is that each affected family has a novel mutation.
Another diagnostic route attempts to identify evidence of abnormal storage through the analysis of urinary excretory products. Thin-layer chromatography is used to separate abnormal oligosaccharides and glycopeptides in the urine, again by means of normal samples as controls.
Although less commonly measured, dogs with fucosidosis have been found to excrete fucoglycoconjugates, whereas cats with mannosidosis have urine containing mannose-rich oligosaccharides. Both enzyme analysis and urinary excretory product analysis require the assistance of a laboratory skilled in the techniques involved.

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