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CLINICAL CASE

A Fatal Case of Generalized Lysosomal Storage Disease in an Infant

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KEY WORDS
Gangliosidoses; lysosomal storage disease; metabolic neurodegenerative disorders.

Abstract
Gangliosidoses are a heterogeneous group of lysosomal storage diseases with an autosomal recessive trait, which are characterized by the intracellular accumulation of gangliosides in several tissues, mainly in neurons. This condition causes a progressive neurodegenerative disorder with varied clinical presentations. Depending on the severity of the enzymatic defect, gangliosidoses show different rates of clinical progression and organ involvement; poor residual enzyme activity is seen in more aggressive forms (infantile and juvenile subtypes) leading to early death whereas cases with better residual enzyme activity have a late onset in adult life and a milder clinical course. Autopsy findings of a 7 month-old girl with histological and ultrastructural changes consistent with gangliosidosis are presented.

PALABRAS CLAVE
Gangliosidosis; Enfermedad de almacenamiento lisosomal; desórdenes neurodegenerativos metabólicos.

Caso mortal de enfermedad de almacenamiento lisosomal en una niña de 7 meses

Resumen
Las gangliosidosis son un grupo heterogéneo de enfermedades de almacenamiento lisosomal que se heredan con carácter autosómico recesivo y se caracterizan por la acumulación intracelular de gangliósidos en distintos tejidos, principalmente en las neuronas. La condición causa un trastorno neurodegenerativo progresivo con presentaciones clínicas variadas. Las gangliosidosis exhiben diferente evolución clínica y compromiso orgánico dependiendo de la gravedad del defecto enzimático; en las formas más agresivas (subtipos infantil y juvenil) se observa escasa actividad enzimática

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residual, lo que conduce a la muerte temprana, mientras que en los casos con mejor actividad enzimática residual la presentación ocurre más tardíamente en la adultez y la enfermedad tiene un curso clínico más leve. Se presentan los hallazgos de la autopsia de una niña de 7 meses con cambios histológicos y ultraestructurales de gangliosidosis.

Case presentation

The patient was a 7 month-old girl product of the third pregnancy of non-consanguineous parents, whose delivery was uneventful. The patient had been initially seen by a pediatrician because of macrocephalia and psychomotor delay; no definitive diagnosis had been made. Family history was negative. The patient was first seen at the pediatric emergency room of our institution because of a febrile respiratory illness. Physical examination, routine lab tests, and a chest x-ray confirmed the diagnosis of pneumonia; rapid influenza A test was positive. The patient was admitted to the pediatrics service where medical management was initiated. During the second day of admission, she suddenly developed vomiting, neurologic collapse, metabolic acidosis, hypotension, and cardiopulmonary arrest, and was unresponsive to resuscitation maneuvers.

A complete autopsy was performed. Weight and height were within normal limits. On external examination, both coarse face and depressed nasal bridge were identified. In situ inspection of the pelvic organs was unremarkable. The lungs showed gross changes consistent with pneumonia. Hepatosplenomegaly was present. The brain weighed 831 g (normal weight according to age/height: 691 g). The most significant gross finding on autopsy was the presence of enlarged convolutions of the frontal and parietal cerebral cortex; sulci also appeared short and scanty (**Figure 1**). These features were more evident on cut surface. The ventricular system, the brain stem, and the cerebellum showed no abnormalities. Except for the eyes, routine representative sections of the organs were submitted for histology. The initial histopathological study demonstrated bilateral acute pneumonia as the direct cause of death. Sections from the central nervous system (CNS), the spinal cord, and sympathetic ganglia from the paravertebral region showed that neurons presented severe morphological changes. The most striking degenerative changes consisted in neuronal enlargement with broad dendritic processes lacking slender cellular borders. The normal appearance of the cytoplasm was replaced by a large amount of granular material leading to swollen neurons with displaced nuclei (**Figure 2**); the intracytoplasmic material was positive for periodic acid-Schiff stain with diastase digestion and luxol fast blue. In addition, both astrogliosis in the brain cortex and spinal cord and ectopic neurons in the cerebellum were seen. Similar cytoplasmic changes were identified in interalveolar and gastrointestinal macrophages. The thymus, the spleen, the liver, and the heart were also affected. Tissue from the CNS and the lungs was submitted for electron microscopy. Ultrastructural study demonstrated numerous whorled membranous and stacked intracytoplasmic structures (**Figure 3**), which represent massive accumulation of glycolipids. Through histological and ultrastructural findings besides the clinical picture, the diagnosis of a lysosomal storage disease affecting the CNS and viscera consistent with infantile generalized gangliosidosis was made.

Discussion

Gangliosidoses are a rare and heterogeneous group of lysosomal storage diseases inherited with an autosomal recessive trait; the metabolic disorder is caused by mutatations in any of the genes encoding enzymes involved in gangliosides metabolism. The deficiency of enzyme activity leads to accumulation of gangliosides and related glycoconjugates in the lysosomes, which causes lysosomal swelling, cellular damage, and organ dysfunction, primarily in the CNS[1,2] but also in viscera.[3,4] In 1942, Klenk identified a new class of carbohydrate-rich glycolipids which he named gangliosides and from which fatty acids, sphingosine, galactose, glucose, and sialic acid were isolated through hydrolysis; the gangliosides also contain hexosamine.[5] Glycosphingolipids, which are essential components of every eukaryotic cell membrane, are synthesized in the endoplasmic reticulum and the Golgi apparatus and reside mainly on the external leaflet of the plasma membrane; they are internalized by endocytosis and are degraded in the lysosomes. Although once considered to be only a structural part of the plasma membrane, these molecules are now known to play important roles in a large number of regulatory events in adult life such as cell-to-cell and cell-matrix interactions. It is also thought that they are involved in many morphogenesis processes including cell signaling, growth, migration, and

Figure 1. Gross photograph of coronal section of the brain; note the broad convolutions and short sulci.

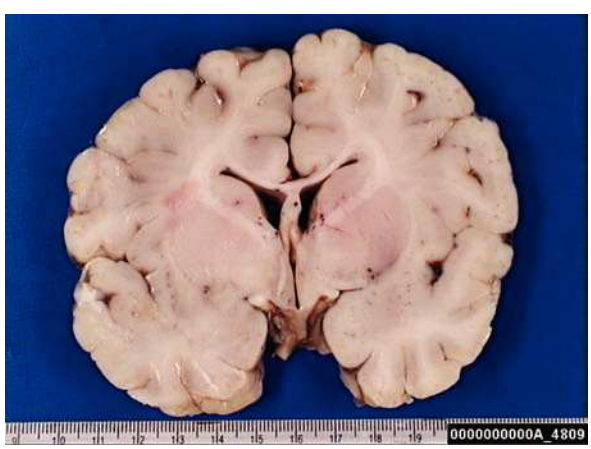


Figure 2. A group of swollen neurons are seen in this picture, gliosis (upper left) and meganeurites also are identified (inset). Hematoxilin & Eosin.

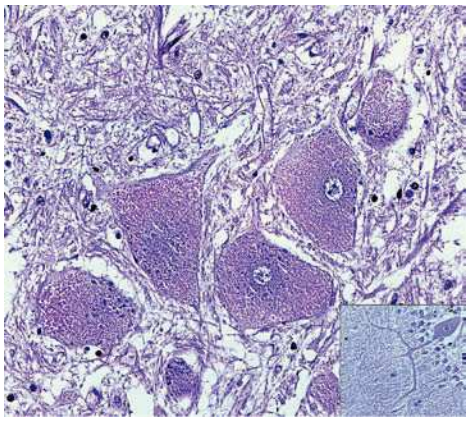
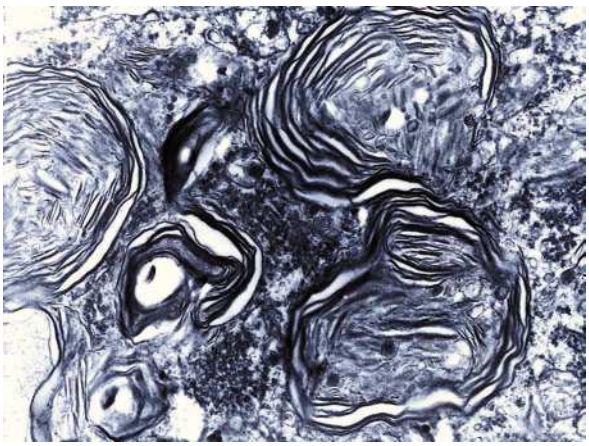


Figure 3. Numerous membranous lamellar bodies are seen in this Ultrastructure.



differentiation.[6] As gangliosides are seen at the highest level of brain tissue, progressive neurological involvement is the rule. Genetic deficiency of lysosomal enzyme is varied; consequently, biochemical changes produce a significant heterogeneity in phenotypic expression. The progression of the disease depends largely on the nature of the mutation and its effect on enzyme activity. Mutations on the gene GLB1 which maps to the short arm of chromosome 3, encode the ß-galactosidase enzyme and cause GM1 gangliosidosis and Morquio B disease,[7,8,9,10] whereas mutations on the HEXA gene (code for a-hexosaminidase subunit-α) and the HEXB gene (code for b-hexosaminidase subunit-ß) produce the GM2 gangliosidoses Tay-Sachs disease and Sandhoff disease, respectively; mutations on the GM2A gene result in GM2 activator deficiency.[11,12,13] Clinical presentation, organ involvement, and age of onset show significant heterogeneity ranging from infantile-onset, rapidly progressing neurological damage, and premature death to late-onset, sub-acute, juvenile-onset, and chronic or adult forms with fewer neurological manifestations (Table 1).[1,14-16] The severity of each subtype is inversely related to the residual activity of the mutant enzyme.[1,11] However, the sine qua non of the disease is the progressive neurodegenerative damage that may culminate in death in the most aggressive forms of the disease.[17] Gangliosidoses are extraordinary diseases for which a panethnic background has been reported; increasing prevalence has been identified in Brazil, Japan, Malta, Cyprus, and among Jewish Ashkenazi and in Roma population.[18,19,20] The incidence of GM1 and GM2 gangliosidoses has been estimated to be 1 in 100,000–200,000 live births[1] and 1 in 222,000-422,000 live births,[2] respectively. The diagnosis of a neurodegenerative disorder with an inherited metabolic background is not simple and relies mainly on a high index of suspicion and the clinical experience since most cases present with common pediatric problems such as recurrent vomiting, failure to thrive, seizures, developmental delay, and recurrent infections; nevertheless, several clinical features may suggest a lysosomal storage disease (Table 2).[1,15,21] Lysosomal storage diseases share a common feature i.e., the biochemical disorder resulting in the accumulation within the lysosomes of normally degraded substrates. Skin and suction rectal biopsies are useful tools to elucidate the ultrastuctural nature of the accumulated substrate.[22-24] A definitive diagnosis is reached through enzyme assays and molecular biology.[16,25] Imaging may also positively contribute to the diagnosis.[26]

In the described case, clinical features of the patient were mild; however, post-mortem findings including light and electron microscopy were unequivocal. The neurons of the CNS appeared swollen with accumulation of glycolipids; a mild inflammatory process, gliosis, meganeurites, and neuronal ectopia were all observed. The histological changes seen in this case have been reported in the literature and are thought to account for the neuronal dysfunction. Despite this, the underlying mechanisms of the disease are not completely understood. Neuronal apoptosis, astrogliosis, microgliosis, demyelination, abnormal axoplasmic transport, and alteration in neuronal-oligodendroglial interactions have all been proposed as possible mechanisms of pathogenesis.[27] More recently, the inflammatory response has been thought to contribute to the pathogenesis and disease progression.[28,29] In animal models the accumulation of gangliosides activates microglia, which in turn recruit macrophages and T-lymphocytes initiating the inflammatory response.[30] Gangliosidosis is currently an incurable disease and only support therapy is available; different therapeutic strategies have been explored including enzyme replacement, substrate deprivation, bone marrow transplantation, and gene therapy.[31-33] Experiments in animal models using gene therapy and viral vectors have brought new hope for these patients.[34,35] In the present case, the clinical and laboratory diagnostic approach was not completed since the patient had been admitted because of an acute respiratory disease and a lysosomal storage disease was not

Table 1. Clinical, molecular and microscopic features of GM1 and GM2 gangliosidoses.

Disease	Enzyme	Gene	Phenotype	Pathology
GM1 Gangliosidosis Infantile type I Juvenile type II Adult type III	β-Galactosidase	GLB1	Macro/microcephalus, dysmorphic face, melanocytosis, skeletal defects, hypotonia, macular cherry-red spot, hepatosplenomegaly, psychomotor delay. Death before age 4 Less dysmorphic features, slow progression of neurological symptoms, ataxia, dysarthria, seizures, no visceral involvement, lifespan 3-10 years Normal early neurological development, no stigmata, onset of neurological symptoms in adolescence, speech and gait disturbances, dystonia, Parkinson, dementia	Vacuolated lymphocytes, macrophages and neurons, gliosis, inflammation, membranous lamellar cytoplasmic bodies identifiable by ultrastructural imaging
GM2 Gangliosidoses Tay-Sachs disease Acute infantile Juvenile sub-acute Adult chronic Sandhoff disease Infantile Late juvenile and adult GM2 activator protein deficiency	β-hexosaminidase A β-hexosaminidase AB GM2 activator protein	HEXA HEXB GM2A	Neurological symptoms by age 3-6 months, exaggerated startle, abnormal psychomotor development, macular cherry-red spot, blindness, deafness, life span 4-5 years Progressive neurologic deterioration, loss of motor and spinocerebellar functions, gait and speech impairment Normal neurologic development, spinocerebellar degeneration, dystonia, ataxia, atrophy, psychiatric symptoms Similar to Tay-Sachs disease, hepatosplenomegaly, skeletal abnormalities Adult onset, slow progression, postural dystonia, seizures Clinical course similar to infantile Tay-Sachs disease	Vacuolated neurons, gliosis, meganeurites, membranous lamellar/stacked cytoplasmic bodies identifiable by ultrastructural imaging

Table 2. Clinical features associated to neurodegenerative disorders with metabolic background.

Dysmorphic features present at birth	Coarse face, depressed nasal bridge, craniofacial and limb dysostosis, low-set ears, corneal clouding, macular cherry-red spot
Central nervous system	Delay/arrest of psychomotor development, neurological regression, seizures, blindness, deafness, distonia, extrapyramidal/ spinocerebellar symptoms, psychiatric problems
Systemic findings	Bone marrow depression, hepatosplenomegaly, vacuolated lymphocytes, repeated infections, cardiomyopathy, frequent vomiting, constipation, failure to thrive, skeletal abnormalities

suspected; however, from an anatomopathological point of view, a differential diagnosis was carried out. Lysosomal storage diseases (affecting the CNS and viscera) other than gangliosidoses such as Niemann Pick disease and Gaucher's disease were ruled out since the clinical picture, the extension of the disease, and the histological and ultrastructural features were more consistent with a gangliosidoses.

Conclusion

Gangliosidoses are inborn errors of metabolism resulting from the defective activity of degradative enzymes in lysosomes and causing a neurodegenerative disorder. The diagnosis relies on clinical suspicion, imaging, light and electron microscopy, and enzyme assays or molecular biology. Reaching a correct diagnosis is of utmost importance for appropriate therapy and genetic counseling.

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