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Case Report

Ethylmalonic Encephalopathy: A Cause of Petechiae without Alteration in Platelet or Coagulation Tests

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Abstract

Ethylmalonic encephalopathy (EE) is an autosomal recessive disease, rare, whose origin is given by mutations in the gene ETHE1, which codes for the protein of the same name and acts as a sulfur dioxygenase converting it into non-dangerous components such as thiosulfate and sulfate. The mutations generate an accumulation of hydrogen sulfide (H_2S), which at high concentrations acts as an inhibitor of cytochrome c oxidase (COX) and acylCoA. Its main sites of accumulation are brain, liver, smooth muscle and colonic mucosa. The deficit of COX produces high levels of lactate in the blood and accumulation of ethylmalonic acid with the consequent excretion of the latter in urine (ethylmalonic aciduria) and, on the other hand, the decrease in short chain acylCoA dehydrogenase causes an increase in C4-C5 acylcarnitines. Biochemically, the disease is characterized by plasma lactate elevation, methylmalonic aciduria, altered levels of C4-C5 acylcarnitines, cytochrome c oxidase deficiency (COX) and increased thiosulfate concentration. Clinically, the affected systems are central nervous system (CNS), gastrointestinal and peripheral vessels, manifesting with neurological alterations characterized by progressive encephalopathy, chronic diarrhea, petechiae and acrocyanosis. The disease is of early onset, and in most patients the disease is severe with death occurring in the first years of life. So far there is no cure for the disease, but treatments that improve clinical manifestations and survival of patients, being useful N-acetylcysteine and metronidazole, which reduce H2S concentrations.

Keywords: *Ethylmalonic Encephalopathy; Hydrogen Sulfide; Ethylmalonic Aciduria; Progressive Encephalopathy Petechia Acrocyanosis*

Introduction

Ethylmalonic encephalopathy (EE) is an autosomal recessive disease caused by mutations in the ETHE1 gene [1-4] and is characterized by accumulation of hydrogen sulfide (H_2S) [1,4-6]". From the biochemical point of view there is an elevation of plasma lactate, methylmalonic aciduria, altered levels of acylcarnitines (C4-C5), deficit of cytochrome c oxidase (COX) and increase in thiosulfate concentration [1-4,7-10]. The affected systems are central nervous system (CNS), gastrointestinal and peripheral vessels [3,7,9,11], manifesting with neurological alterations with progressive encephalopathy, chronic diarrhea, petechiae and acrocyanosis [2,3,5]. The disease is of early onset and in the most patients the disease is serious, and death occurs in the first years of life.

Case Report

Male patient, 8 months old with an antecedent of mild perinatal asphyxia who required neonatal conduction adaptation, with neurodevelopmental delay, who at 2 months old begins to present consistent paroxysmal events in abduction of upper limbs and flexion of the

head, between 5 and 10 episodes a day; later, the episodes changed, characterized by lateralization of the head to the right with ipsilateral tonic-clonic movements, approximately twice a week and then with supraversion of the gaze and flexion of the extremities. Also, with diarrheal stools from 3 months old. He also presented episodes of intermittent cyanosis in the extremities. He was admitted to the hospital for a fever of 3 days and physical examination revealed generalized hypotonia of axial predominance, without cephalic control, without contact with the environment, with generalized petechial skin lesions and a hematoma in his left forearm.

No dysmorphic facies were observed.

Consanguineous parents, without significant family history.

He was evaluated by ophthalmology without finding alterations.

Paraclinical:

- Electroencephalogram: slow background tracing and multifocal irritative activity.
- Brain magnetic resonance with hyperintensity of the left globus pallidus and gliosis.
- Ammonium 31.
- GPT 17 IU/L, GOT 32 IU/L, FA 205 U/L
- Creatinine 0.38 mg/dl, BUN 8.1 mg/dl.
- Sodium 138 mEq/L, potassium 4.2 mEq/L, chloride 117 mEq/L, calcium 9.3 mg/dl.
- Venous gases: pH 7.28, pCO_2 33, pO_2 42, HCO_3 15, lactate 2.8 (metabolic acidosis with hyperlactatemia).
- Ketones in blood 0.9.
- Normal uroanalysis.
- Hemoglobin 10.7, hematocrit 32%, leukocytes 5.900, neutrophils 2.200, lymphocytes 3.400.
- Platelets 339.000, TP 13 (control 10), TPT 37 (control 30).
- Ultrasonography of the abdomen: Liver with discrete increase in diffuse form of its size, left hepatic lobe measures 50 mm and right 82 mm that suggests a hepatic steatosis.
- Doppler of upper and lower arterial circulation: Permeability without stenosis.

Due to suspect inborn error of the metabolism with convulsive syndrome, management was started with phenobarbital/midazolam and later vigabatrin was added, and cofactors: L-carnitine, pyridoxine, thiamin, folic acid and riboflavin.

Due to the association of the manifestations of the clinical picture, organic aciduria, petechiae and skin ecchymoses, acrocyanosis and chronic diarrhea, it was considered an EPEMA syndrome. A few days after admission to the hospital the child died and it was not possible to make an accurate diagnosis of the disease.

Discussion

EE is an autosomal recessive disease caused by mutations in a gene known as ETHE1 (Ethylmalonic Encephalopathy 1) [1-4] and it is also called EPEMA syndrome (Encephalopathy, Petechiae and Ethylmalonic Aciduria) [12], characterized by accumulation of H_2S [1,4-6]. It is a rare disease, being more common in Mediterranean and Arabian populations [3,7,13], although there have been documented cases of patients all over the world.

ETHE1 is a gene located on chromosome 19q13 [1,3] which codifies for the ETHE1 protein that is a thioesterase of the mitochondrial matrix and acts as a sulfide dioxygenase [2-4] being part of the oxidative pathway that converts the sulfide into non-dangerous components such as thiosulfate and sulfate. They have been described more than 20 mutations [8] that can be of different types such as missense, nonsense, alterations in the reading frame and deletions [5], generating the greatest loss of function the ETHE1 protein

However, they have been identified some missense mutations that affect amino acid residues highly conserved and is associated with the presence of the protein in normal or decreased amount [1,13]. It has not been observed that the type of mutation has any correlation with the clinical manifestations of the disease [13].

The H_2S and its role in the EE

H_2S is a water- soluble and colorless gas. In high concentrations it is a potent inhibitor of cytochrome c oxidase, whereas in small quantities it is an important signaling molecule [5]. Within their functions, they act as neuromodulator [1,5,14] increasing the production of cAMP and facilitating the induction of long-term potentiation by increasing the activity of N-methyl-d-aspartate (NMDA) receptors in hippocampal pyramidal neurons [1,5]. It also has action on active synapses, which is why it is believed to have a role in associative learning [1]. It is a component that produces vasodilation [1,5,14] because it activates the potassium channels that depends on ATP, generating higher membrane potential in muscle cells at the vascular level, which are hyperpolarized and therefore produces vasorelaxation [5]. It also induces the proliferation of smooth muscle cells stimulating angiogenesis [1,5,7] and has an effect on the regulation of antioxidant systems such as N - acetylcysteine and glutathione and in the positive regulation of anti-inflammatory genes and cytoprotectives [5].

H_2S is produced endogenously in the cytoplasm by the desulfurization of cysteine or homocysteine through the transulphurisation enzymes cystathionine ß-shyntase and cystathione y-lysa [1,5,8,9]. Cystathionine ß-synthase is expressed predominantly in SNC, but is also found in liver and kidneys, while the cystathionine y-lyase is found primarily in liver and vascular smooth muscle and nonvascular as small intestine and stomach [5]. A third enzyme, the 3-mercaptopyruvate sulfurtransferase (MST) can also contribute to the production of said compound [1,8]. Additionally, the H_2S can be formed from the anaerobic enteric flora, counting the intestinal epithelium with systems of specialized enzymes that convert the H_2S into thiosulfate and sulfate, thus preventing the local increase to toxic levels and its entry through the portal venous system to the liver and other organs [1,5]. A last origin of H_2S is the non-enzymatic reduction of elementary sulphur, although it is not so important [5].

The catabolism of H_2S occurs in the mitochondria together with the electron transfer chain [9,15] and consists of a series of oxidative reactions that elevate the thiosulfate and sulphite production as intermediate components [5,8]. In the process, the quinone sulfide oxidoreductase enzyme binds to the mitochondrial membrane and leads to the initial oxidation of H_2S and the fixation of sulfur atom to an acceptor of sulphur forming persulphite, then the persulphite is transferred to glutathione or other acceptor that has action ETHE1 (dioxygenase of sulphur) and is converted to sulfite, which by action of the rhodanese enzyme is trans -sulfurated turning it into thiosulfate [4,9].

Mutation in the ETHE1 gene leads to accumulation of H_2S in brain, liver, smooth muscle and colonic mucosa [1,4,5] in amounts that inhibit the COX and acylCoA dehydrogenase [1,4,5,8,16]. In mice with gene mutations, elevation of H_2S concentrations has been observed up to 20 times more than normal [8]. The chronic inhibition of COX leads to structural destabilization with accelerated degradation of it [4,5]. The deficit of COX produces high levels of lactate in blood and accumulation of ethylmalonic acid with the consequent excretion of the latter in urine (methylmalonic aciduria) [5]. The ethylmalonic acid is an organic dicarboxylic acid produced by the carboxylation of butyrate and is the trigger of enzymatic defects of ß- oxidation of fatty acids and branched-chain amino acids, which is why in patients with EE there is accumulation of C4-C5 acylcarnitines due to deficiency of acylCoA dehydrogenase short chain [5].

It is thus, as biochemically the disease is determined by the presence of elevated lactate in blood, methylmalonic aciduria, altered levels of C4-C5 acylcarnitines, COX deficit and increase in thiosulfate concentration [1-4,7-10], which is also elevated in urine [7].

It is believed that the reasons why the thiosulfate is high in patients with EEM are: 1. is an alternate substrate for ETHE1, reason why is accumulate in patients with EEM [9] and 2. in absence of functional ETHE1, the persulphite produced by quinone oxidoreductase sulfur that is consumed preferentially by rhodanese to form thiosulfate in large quantities [8,9,17].

Clinical manifestations of the EE

It is characterized by a set of symptoms and signs, which may not all be presented together, and although in most patients the disease is severe and death occurs in the first two years of life, some may have a mild symptomatology with a chronic course [2]. The syndrome is

of an early onset, being able to present from the neonatal period in 25% of cases [10] or in the first months of life, and apparently there is no direct relationship between the time of onset and the subsequent clinical evolution [12]. The main affected organs are the brain, gastrointestinal system and peripheral blood vessels [3,7,9,11], manifesting itself with neurological disorders such as hypotonia, limitation in achieving development goals or stopping them in children with normal neurodevelopment prior to the onset of symptoms, seizures, movement disorders and progressive encephalopathy, chronic diarrhea, petechiae and acrocyanosis [2,3,5].

Vascular damage by endothelial accumulation of H_2S is important, with alteration in nitric oxide production [14] and is the cause of petechiae, which are not associated with thrombocytopenia, or alterations in coagulation tests [3,5]. Giordano and cols [16] reported the autopsy of a child with EE genetically confirmed and they found endothelial lesions of arterioles and capillaries of the brain and gastrointestinal tract; also in gastric and colonic mucosa was observed petechial hemorrhages and histological analysis showed multiple hemorrhages in capillaries and venules located in the mucosa, submucosa and in the own muscularis, the kidneys showed diffuse microthrombi in glomeruli and focal glomerulosclerosis and in the brain were found luminal microthrombi, acute microhemorrhages and macrophages loaded with hemosiderin which indicated recent bleeding.

In brain magnetic resonance its observed hyperintensity in T2 phase in putamen, caudate nucleus and periventricular region [3,12,16,18,19]. They may also have abnormalities in subcortical areas, white matter and brainstem and in some frontotemporal hypoplasia [3,12], atrophy of cerebellar hemispheres and vermis [12]. The lesions found indicate the presence of secondary necrotic lesions to the toxicity of accumulated ethylmalonic acid, or vascular changes [12,19]. Similarly, some children may have malformations as Chiari type 1 and of the spinal cord [18].

Although the characteristics already mentioned are the main, they have been described case reports of patients with involvement of other organs such as kidney with evidence of rapidly progressive glomerulonephritis [20], hydronephrosis and undescended testes [21], and consisted dysmorphic facial characteristics in epithelial folds and broad nasal bridge [3]. As previously mentioned, not all children who have the syndrome have the typical characteristics, in fact, Pigeon and cols [18] reported the case of monochorionic twins who at 10 years old had not presented petechiae, acrocyanosis or chronic diarrhea, but both had developed pyramidal syndrome, indicating the heterogeneity that can be seen in the disease.

Treatment of EE

As previously mentioned, most children with the disease die during the first 2 years of life, and while there is no cure for the disease, different treatments have been found that improve the clinical manifestations and survival of these patients.

It has been found that N-acetylcysteine and metronidazole decrease H_2S concentrations. The first one is an intracellular precursor of glutathione and in the mitochondria acts as a sulfide acceptor through the quinone sulfide oxireductase. For its part, metronidazole decreases bacteria at the intestinal level, thus reducing H_2S levels. In mice with ETHE1 mutation and in patients with EE it has been found that the combination of these two components improves vascular lesions and diarrhea, as well as decreases some neurological abnormalities [1].

Cell therapy studies have also been carried out in mice with the mutation, in which vectors associated with adenovirus serotype 8 have been injected, which have tropism by hepatocytes and which contain the ETHE1 gene, with recovery of enzymatic activity, restoration of the biochemical profile and greater survival [1].

Conclusions

Ethylmalonic encephalopathy is a rare disease, characterized by an accumulation of hydrogen sulfide caused by mutations in the ETHE1 gene that codes for a protein of the same name and whose role is to convert hydrogen sulfide into non-toxic components. The

accumulation of this product generates inhibition of COX with the consequent increase in lactate and ethylmalonic acid in plasma, and also inhibits acylCoA, causing in turn an increase in C4-C5 acylcarnitines. The disease is of early onset and affects the central nervous system with delayed or delayed neurological development, hypotonia, seizures, movement disorders and progressive encephalopathy. It also causes vascular injury with alteration of nitric oxide, which causes petechiae that do not relate to alteration in the coagulation tests, there is acrocyanosis and damage in colonic mucosa with chronic diarrhea. The disease has no curative treatment, but it does have support to diminish the clinical manifestations and improve the survival of the patients, having demonstrated the utility of N-acetylcysteine and metronidazole that decrease the concentrations of hydrogen sulfide. It is a rare disease, which is why you should have a high index of suspicion in children who meet the mentioned characteristics.

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