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CASE REPORT

Involvement of mitochondrial dysfunction in pathogenesis of hemophagocytic lymphohistiocytosis

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ABSTRACT

Background: Hemophagocytic lymphohistiocytosis (HLH) is a hyper-inflammatory condition resulting from altered crosstalk between innate and adaptive immune responses. Familial HLH is caused by mutations in several genes whereas the acquired form is associated with infections, rheumatic diseases, malignancies, and inherited metabolic disorders.

Case Presentation: We report an infant boy who developed HLH and the potential involvement of mitochondrial DNA in pathogenicity. This patient was with evidence of mitochondrial disease based on neonatal-onset lactic acidosis, elevated lactate in cerebrospinal fluid, a significant lactate peak on magnetic resonance spectroscopy, and generalized reduction of multiple respiratory chain enzyme complex activities. In addition, full mitochondrial genome sequencing only revealed the identification of the homoplasmic m.4325A > G mutation. Subsequently, he presented with a febrile illness complicated by HLH.

Conclusion: Elevated levels of lactic acidosis and mitochondrial dysfunction strengthen the involvement of mitochondria in causing secondary HLH in our patient.

Keywords: Hemophagocytic lymphohistiocytosis, mitochondrial dysfunction, inherited metabolic.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) represents a group of potentially fatal disorders that are rapidly progressive and characterized by exaggerated hyper-inflammatory responses (1). The clinical manifestations of HLH include fever, splenomegaly, pancytopenia, biochemical and immunological disturbances, and hemophagocytosis into bone marrow, liver, or lymph nodes. HLH is classified in two forms: primary/familial (OMIM 267700) and secondary/reactive sporadic HLH (2). Since 1999, at least four genes (*PRF1*, *MUNC13-4*, *STX11*, and *STXBP2*) have been shown to cause familial HLH, while a number of genes associated with other abnormalities, including *SH2D1A* and *BIRC4* (X-linked lymphoproliferative syndrome), *RAB27A* (Griscelli II), *LYST* (Chediak-Higashi syndrome), and *AP3B1* (Hermansky-Pudlak II) have been linked to higher incidence of HLH. Secondary causes of HLH include infections, collagen-vascular diseases, inherited immune deficiencies, malignancies, and inborn errors of metabolism (3,4).

Recently, HLH was described to be caused by a defect in the mitochondrial respiratory chain system (5). Herein, we report an infant boy who developed HLH with evidence of mitochondrial cytopathology.

Case Presentation

This infant boy was born at term to unrelated parents. The family history was significant for three neonatal/infantile deaths of unknown cause. He was normal upon physical examination. The renal and liver function tests and a complete blood count were typical. However, he had elevated serum lactate at 4–8.8 (reference: 0.5–2 mmol/l), but ammonia, MS/MS acylcarnitines, serum biotinidase, and urine organic acid analysis were unremarkable. At

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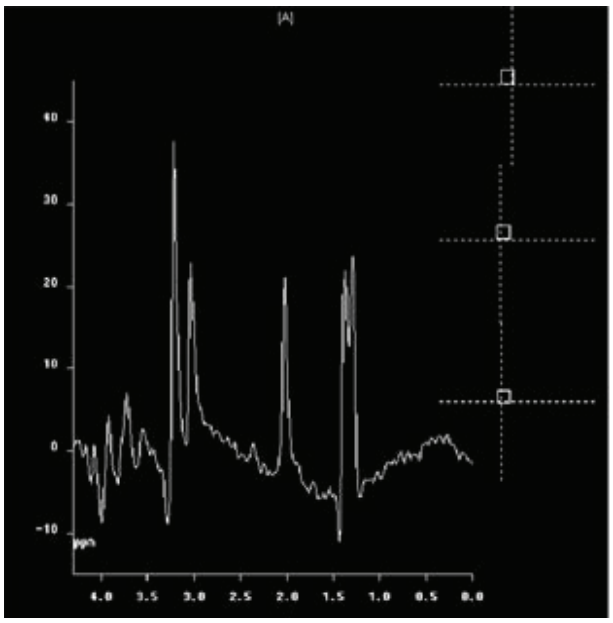

Figure 1. *MRS of the brain image shows increased lactate peak.*

the age of 4 weeks, he had a febrile illness associated with hepatosplenomegaly. Central and axial hypotonia was noted. He was treated empirically with antibiotics. Blood, urine, and cerebrospinal fluid (CSF) cultures were normal. The patient deteriorated with respiratory distress and severe lactic acidosis (lactate > 23.3 mmol/l). The CSF lactate was also high, at 3.5 (reference: 0.5–2 mmol/l). A brain magnetic resonance imaging was unremarkable but magnetic resonance spectroscopy revealed a large lactate peak in the basal ganglia (Figure 1). The patient required mechanical ventilation. He had developed focal seizures that were treated with phenobarbital and topiramate. Pertinent investigations are shown in Table 1. His CSF red blood cell, white blood cell (WBC) and protein which were 91 (reference: 0×10^6/l), 6 (reference: 0–5 $\times$ 10^6/l), and 3,407 (reference: 150–450 mg/l), respectively. Serum immunoglobulins and blastogenesis were normal, as were plasma and urinary amino acid concentrations of lysine, ornithine, and arginine.

The patient was treated according to HLH 2004 protocol, including intrathecal therapy. He showed improvement with normalization of blood counts, triglycerides, fibrinogen, and ferritin. At the age of 6 months, he was in remission. He was able to roll from side to side with good eye fixation and fair head control, and the spleen was not palpable. Treatment with coenzyme Q, thiamine, carnitine, vitamin K, and riboflavin was not effective in controlling the lactic acidosis. At the age of 7 months, he presented with respiratory distress associated with coagulase-negative staphylococcal sepsis. His status deteriorated quickly and he succumbed to his illness.

Muscle histopathology revealed prominent lysosomal activity, while electron microscopy showed increased lysosomes and a focal increase in mitochondrial density. The activities of respiratory chain complexes were as follows: Complex I activity 27%, total complex I + III activity 36%, complex I + III (rotenone-sensitive) activity 30%, complex II activity 15%, complex II + III activity 38%, complex IV activity 36%, and citrate synthase activity 57% of the control.

Screening of the familial HLH nuclear genes (*PRF1*, *MUNC13-4*, *STX11*, and *STXBP2*) was negative. Full MtDNA analysis only identified the homoplasmic m.4325A > G mutation. The parents declined to provide DNA samples for the study.

Discussion

Mitochondrial disorders, a group inherited metabolic diseases, are clinically and genetically heterogeneous conditions, commonly defined by a loss of adenosine triphosphate production due to deficiencies of oxidative phosphorylation. Although dysfunction of the central nervous system is a prominent feature of mitochondrial disorders, multisystem involvement (such as heart, liver, kidneys, eyes, cochlea, and pancreas) is commonly seen (6). HLH is not a single disease but rather a hyperinflammatory syndrome caused by excessive activation of lymphocytes and macrophages that produce high levels of cytokines, and in fact, symptoms of HLH reflect hypercytokinemia (7). HLH has been sporadically reported in a wide range of inherited metabolic disorder (IMD). The association between HLH and IMD is well-recognized, the best example being lysinuric protein intolerance (LPI) (8). HLH has been reported in association with Gaucher disease, mtDNA deletion syndromes, Cobalamin C defect, organic acidemia (propionic acidemia and methylmalonic acidemia), galactosialidosis, and galactosemia (9). Recently, encephalopathy was reported with a manifestation of HLH in a patient with propionic acidemia (10).

Our patient developed HLH fulfilling the diagnostic criteria: fever, splenomegaly, pancytopenia, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, high soluble CD25, and hemophagocytosis in the bone marrow without evidence of malignancy. In addition, he had significant lactic acidosis at birth along with evidence of cerebral lactic acidosis by CSF examination and magnetic resonance spectroscopy (MRS). Mitochondrial dysfunction was confirmed by respiratory chain enzymatic assays showing a deficiency of multiple enzymatic complexes. These metabolic abnormalities point to a mitochondrial disorder; however, the precise molecular etiology of the patient's disease, therefore, remains unidentified. The patient in this report is similar to the neonate reported by Fuwa et al. (5) who developed uncontrollable HLH and was diagnosed with mitochondrial disorder due to mitochondrial complex I deficiency. Another patient with a large mtDNA deletion (~90%) was previously reported with lactic acidosis and infant-onset HLH. He was diagnosed as having "Pearson syndrome," but did not have sideroblastosis in his bone marrow nor did he have evidence of exocrine pancreatic insufficiency (11).

Table 1. *Summary of essential laboratory findings in the patient.*

Hematological studies			
Parameter		**Patient**	**Reference**
WBC (ANC)		4.2 (890)	6–18 × 109/l
Hemoglobin		7.4	14–20 g/dl
Platelets		35	140–350 × 109/l
INR		1.5	0.86–1.14
PT		18.5	11.9–14.3 seconds
PTT		49	34.7–42.2 seconds
Fibrinogen		90	150–400 mg/dl
AST		6340	10–45 U/l
ALT		1217	10–45 U/l
Triglycerides		292	0–150 mg/dl
Ferritin		25,039	30–400 ng/ml
CD25		>5,000	458–1,997 pg/ml
CSF studies			
CSF WBC		31	0-5 x10.6
CSF protein		68	150-450 mg/L
Mitochondrial respiratory chain complex activities			
		Patient (% of mean*)	Control ± SD (nmol/min/mg protein)
Complex I		5.64 (27)	20.74 ± 8.73
Complex I + III	Total	1.78 (36)	4.96 ± 2.15
	Rotenone-sensitive activity	0.75 (30)	2.52 ± 1.28
Complex II		0.18 (15)	12.00 ± 0.27
Complex II + III		0.45 (38)	1.19 ± 0.46
Complex IV		1.24 (36)	3.40 ± 1.38
Citrate synthase		5.41 (57)	9.57 ± 4.72

The electron transport chain enzymes were assayed using a temperature-controlled spectrophotometer.

*The second figures in parentheses represent data after normalization against citrate synthase.

The pathogenesis of HLH in IMDs is still unclear. Mechanisms in acquired HLH are probably diverse and may have to act in combination (7). There is often some involvement of the bone marrow in IMDs associated with HLH. For example, bone marrow depression can sometimes be seen after a metabolic crisis such as in propionic acidemia and methylmalonic acidemia (12). Bone marrow infiltration by storage/foam cells is seen in lysosomal storage disorders such as Gaucher disease and galactosialidosis. Sulfatase modifying factor 1, the defective protein in multiple sulfatase deficiency, was shown to control hematopoietic stem/progenitor cell differentiation and hematopoietic lineage development (13). Finally, there may be an increased risk for viral and bacterial infections in patients with organic acidemia that could possibly act as a trigger for HLH. Among these metabolic disorders, LPI is the best studied example. LPI is caused by defective *SLC7A7* gene leading to cationic amino acid (arginine, lysine, and ornithine) transport disturbance at the epithelial cells of intestine and kidneys. Barilli et al. demonstrated that the mutation of SLC7A7/y + LAT1 strongly impaired arginine efflux through system y + L in LPI macrophages. Thus, suggesting that intracellular arginine concentration might be abnormally increased leading to the generation of a large amount of nitric oxide (NO) and, in turn, of peroxynitrites (ONOO−) that would act as cytotoxic agents (14).

In mitochondrial disorders, both respiratory chain complexes I and III in mitochondria are the major sources of superoxide and H_2O_2 in mitochondria (15) and our patient was confirmed to have biochemical defects in those complexes, this raises the possibility that such defects may be accompanied by reactive oxygen species production causing oxidative stress, which may cause HLH as a by-product. This strengthens the involvement

of mitochondrial respiratory chain complexes defect in developing HLH as a similar case was recently described (5).

Conclusion

We described a patient with elevated levels of lactic acidosis and mitochondrial dysfunction. This strengthens the involvement of mitochondria in causing secondary HLH in our patient regardless of the exact mechanism. In addition, the measurement of mitochondrial respiratory chain enzymes activity should be considered while investigating patients suspected with mitochondrial disease and secondary HLH.

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Declaration of conflicting interests
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Ethical approval
Ethical approval is not required at our institution to publish an anonymous case report.

Consent for publication
Informed consent was obtained from the parents.

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