Case Report
Mitochondrial Disease as a Cause of Neonatal Hemophagocytic Lymphohistiocytosis

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Received 22 June 2016; Accepted 22 August 2016

Academic Editor: Daniel K. L. Cheuk

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Diagnosis of mitochondrial respiratory chain disorder (MRCD) is often difficult. Its pathogenesis is still unclear. We diagnosed MRCD by measuring the activity of the mitochondrial respiratory chain enzyme, and the patient also had hemophagocytic lymphohistiocytosis (HLH). A preterm female infant was born at 34 weeks of gestation. On day 6, HLH was revealed by bone marrow aspiration. She died on day 10 due to uncontrollable HLH. An autopsy was performed, and we measured the activity of the mitochondrial respiratory chain enzyme in the liver, muscle, and heart. The activity of complex I was decreased in all tissues. As we could not prove another origin of the HLH, she was diagnosed as having HLH caused by MRCD. It is useful to measure the activity of the mitochondrial respiratory chain enzyme for diagnosing MRCD. MRCD, which has a severe clinical course, may be related to HLH.

1. Introduction

Mitochondrial respiratory chain disorders (MRCDs) are dysfunction of the oxidative phosphorylation. MRCD is the most frequently congenital metabolic disease. The frequency is 1/5000 births. Half of MRCD cases are diagnosed in the neonatal period [1]. MRCDs in the neonatal period are fatal and show severe syndromes that are derived from multiple organs. Therefore, it is difficult to diagnose MRCD in the neonatal period. These pathogeneses are still unclear [2].

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory disorder in an uncontrolled and ineffective immune response. HLH occurs either as a primary form or as a secondary form [3]. MRCDs as a cause of secondary form HLH are very rare.

We could diagnose MRCD by measuring the activity of the mitochondrial respiratory chain enzyme. We encountered a preterm infant who had MRCD that might have caused HLH. In this paper, we discuss the way to diagnose MRCD and the correlation between MRCD and HLH.

2. Case Report

A 39-year-old woman with no gestation and no parturition became pregnant by intracytoplasmic sperm injection. There was no family history of sudden death or consanguineous marriage. At 34 weeks and 4 days of gestation, fetal hydrops (pleural effusion, ascites, and subcutaneous edema) was recognized. The infant was delivered by emergency caesarean section due to a nonreassuring fetal status. The infant was intubated due to grunting and transferred to our NICU. Apgar scores were 7 (1 min)/9 (5 min). Her weight was 2264 g (appropriate for dates). Her muscle tone was low. Mild retractions with no murmur and no rale were recognized. Abdominal finding was the distention with hepatosplenomegaly. No abnormal findings existed. We show laboratory findings in Table 1. Lactic acidosis had continued, and jaundice appeared within 24 hours of birth. Disseminated intravascular coagulation (DIC) was recognized. The ammonia, C-reactive protein, and ferritin levels increased. On day 1, we measured cytokines. The findings were the
Table 1: Laboratory findings on admission.

| CBC   |            |            |            |            |
|-------|------------|------------|------------|------------|
| WBC   | 25.3 × 10³/µL |            |            |            |
| Band  | 10%        |            |            |            |
| Seg   | 28.5%      |            |            |            |
| Lymph | 47%        |            |            |            |
| Mono  | 11%        |            |            |            |
| Eosino| 1.0%       |            |            |            |
| Baso  | 0%         |            |            |            |
| Blast | 0%         |            |            |            |
| RBC   | 2.34 × 10⁶/µL |            |            |            |
| Hb    | 8.3 g/dL   |            |            |            |
| Ht    | 25.1%      |            |            |            |
| Plt   | 1.7 × 10⁴/µL |            |            |            |
| Ret   | 54.1‰      |            |            |            |

| Coagulation |            |            |            |            |
| PT          | 37.1 sec   |            |            |            |
| APTT        | 110.7 sec  |            |            |            |
| Fib         | 40 mg/dL   |            |            |            |
| HPT         | 22.6%      |            |            |            |
| FDP         | 25.1 µg/dL |            |            |            |
| AT3         | 8.8%       |            |            |            |

| Biochemistry findings |            |            |            |            |
| T-Bil            | 7.7 mg/dL  |            |            |            |
| D-Bil            | 0.2 mg/dL  |            |            |            |
| AST              | 47 IU/L    |            |            |            |
| ALT              | 61 U/L     |            |            |            |
| CK               | 69 IU/L    |            |            |            |
| BUN              | 7 mg/dL    |            |            |            |
| Cr               | 0.61 mg/dL |            |            |            |
| Na               | 138 mmol/L |            |            |            |
| K                | 4.2 mmol/L |            |            |            |
| Cl               | 109 mmol/L |            |            |            |
| Ca               | 9.3 mg/dL  |            |            |            |
| P                | 5.7 mg/dL  |            |            |            |
| TP               | 3.2 g/dL   |            |            |            |
| ALB              | 2.0 g/dL   |            |            |            |
| NH₃              | 156 µg/dL  |            |            |            |
| Ferritin         | 3701.9 ng/mL |        |            |            |
| CRP              | 1.23 mg/dL |            |            |            |

Blood type: O Rh(+)  
Immunological  
IgG: 333 mg/dL  
IgA: 0 mg/dL  
IgM: 12 mg/dL  
Direct Coombs test: (−)  
Peripheral smear: Spherocytosis: (−)  
Elliptocytosis: (−)  

Blood gas analysis (artery)  
HCO₃⁻: 12.9 mmol/L  
BE: −10.8 mmol/L  
Lac: 70.1 mg/dL  
Anion gap: 16.1

Chromosomal test (G band): 46,XX

This table shows all laboratory findings on admission. Bold font: high; italic font: low.

Following: IL-6 level 1398.3 pg/mL, IL-8 21675.0 pg/mL, INF-γ 5 pg/mL, and TNF-α 8.2 pg/mL. The findings showed hypercytokinemia. We performed an exchange transfusion three times (twice on day 1 and once on day 2) for the early onset jaundice. The patient had prolonged lactic acidosis, mild hyperammonemia, and liver failure that was similar to the presentation of Reye syndrome. Laboratory change, which is related to liver failure, is shown in Figure 1(a). Neonatal mass screening by tandem mass spectrometry, amino acid analysis, and urine organic acid analysis showed normal findings. From the above results, we suspected mitochondrial disease and began to administer vitamin B1, vitamin B2, vitamin C, Carnitine, and Coenzyme Q10 on day 2. On day 5, in the blood, lactate was 39.0 mg/dL, and pyruvic acid was 1.16 mg/dL (lactate/pyruvic acid ratio of 33.2 > 20). In the cerebral spinal fluid, lactate was 43.6 mg/dL, and pyruvic acid was 1.73 mg/dL (lactate/pyruvic acid ratio of 25.2 > 20). These results were also suggesting that she was mitochondrial disease. Thrombocytopenia and anemia did not improve. Specifically, thrombocytopenia was severe, and the patient received continuous platelet transfusion (Figure 1(b)). On day 6, we performed bone marrow aspiration, because mitochondrial disease was not enough for explaining pathogenesis of thrombocytopenia. We recognized a large amount of hemophagocytic macrophages, and we could not detect any evidence of malignancy. She was afebrile. However, the splenomegaly continued from admission. Other examination findings were as follows: WBC 11.4 × 10⁵/µL, Hb 7.9 g/dL, Plt 2.4 × 10⁴/µL, fibrinogen 74 mg/dL, and soluble IL-2 receptor 3805 U/mL. Although we could not measure fasting triglycerides and NK cell activity, our case was diagnosed as hemophagocytic lymphohistiocytosis (HLH), because she had five criteria of Revised Diagnostic Guidelines for HLH [4]. We began to administer prednisolone (2 mg/kg/day) on day 6 and cyclosporine (1 mg/kg/day) on day 8 to treat HLH. We also administered immunoglobulin (500 mg/kg) on day 8. On day 10, the patient died due to uncontrollable HLH, and an autopsy was performed after parental consent was given to perform the autopsy on the whole body except for her brain.

We took samples from the liver, muscle, and heart, and they were preserved at −80°C. Activities of the mitochondrial respiratory chain complexes I, II, III, and IV were assayed as described previously [5]. In these assays, citrate synthase (CS) was used as a housekeeping mitochondrial enzyme marker. The percent ratio of complex I activity to CS activity was decreased in all tissues (Table 2). We diagnosed MRCD (mitochondrial complex I deficiency) based on the diagnostic criteria given by Bernier et al. [6]. According to the results above, she was diagnosed as having MRCD with HLH. We could not detect an antecedent infection (Table 3). Although the mutation of PRF1 is the most common genetic mutation of primary HLH in Japan, she had no mutation of PRF1.

3. Discussion

We determined two important clinical issues. First, it is useful to measure the activity of the mitochondrial respiratory chain enzyme for diagnosing MRCD. Second, MRCD, which has a severe clinical course, may be related to HLH.
The diagnostic criteria given by Bernier et al. is useful for diagnosing MRCD [6]. This diagnosis requires one of the following: enzymology, histology, functional assay, or molecular assay.

Enzymatic diagnosis of MRCD is achieved by the activity of the mitochondrial respiratory enzyme. We can accurately measure the activity of the mitochondrial respiratory chain enzyme, as we appropriately take a sample and immediately preserve it at −80°C [7]. We can determine the biological function of the mitochondria by measuring the activity of the mitochondrial respiratory chain enzyme. In histology, the fact that there are >2% ragged red fibers in the skeletal
Table 2: Enzyme assay of the mitochondrial respiratory chain (I–IV).

| Patient | % of normal | CS ratio (%) | Co II/CS ratio (%) | Co IV/Co II ratio (%) |
|---------|-------------|--------------|---------------------|-----------------------|
| Liver   | 15          | 13           | 28                  | 46                    |
| Muscle  | 17          | 26           | 86                  | 30                    |
| Heart   | 15          | 14           | 79                  | 26                    |

Table 3: Laboratory findings about antecedent infection.

| Infection          | Blood                          | Urine                     | Feces/Throat          | Primary isolation of virus (day 7) |
|--------------------|--------------------------------|---------------------------|-----------------------|-----------------------------------|
| Toxoplasma         | IgM (EIA) (--), PCR (--)       |                           |                       |                                   |
| Parvovirus B19     | IgM (EIA) (--), PCR (--)       |                           |                       |                                   |
| Rubella            | IgM (EIA) (--), PCR (--)       |                           |                       |                                   |
| Herpes simplex virus | IgM (EIA) (--), PCR (--)     |                           |                       |                                   |
| Cytomegalovirus    | IgM (EIA) (--), PCR (--)       |                           |                       |                                   |
| Epstein-Barr virus | PCR (--)                       |                           |                       |                                   |

This table shows the laboratory findings regarding antecedent infection. We took samples for primary isolation of virus on day 7. The other samples were taken on admission. All findings were negative.

Half of MRCD cases are diagnosed in the neonatal period, and 35% of the patients were diagnosed as having lethal infantile mitochondrial disorders [1]. As the symptoms of MRCD are nonspecific, the diagnosis is sometimes difficult. Therefore, MRCD may be the cause of the cryptogenic neonatal deaths that have severe clinical courses. HLH may be related to MRCD, which has a severe clinical course.

Cases involving both MRCD and HLH are rare, and we have experienced only one case. We must inform neonotolists about MRCD, because onset of MRCD is during the neonatal period. Then a further study of MRCD should be conducted.

4. Conclusion

We recommend that an assay of the mitochondrial respiratory chain enzyme should be performed if mitochondrial disease is suspected. MRCD may be related to the pathogenesis of secondary HLH.

Consent

The parents have consented to the submission of this case report to the journal.

Disclosure

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Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.
Acknowledgments

The authors thank Dr. Kei Murayama (Department of Metabolism, Chiba Children’s Hospital) for measuring the activity of the mitochondrial respiratory chain enzyme, Associate Professor Hirokazu Kanegane (Department of Pediatrics, Tokyo Medical and Dental University Hospital) for detecting genetic mutations of primary HLH, Dr. Hiroshi Kishimoto (Division of Pathology, Saitama Children’s Medical Center) for performing the autopsy, and Dr. Yuki Arakawa (Division of Hematology & Oncology, Saitama Children’s Medical Center) for diagnosing HLH.

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