

Haemophagocytic Lymphohistiocytosis in a Newborn Infant Presenting with Cholestasis: Case Report

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is a rare, fatal disease. Neonatal cholestasis exhibits symptoms similar to those seen in several newborn diseases. HLH is rapidly fatal; therefore, an effective and prompt differential diagnosis is vital. A 10-hour-old newborn with icterus was referred to our clinic. Laboratory examination revealed direct bilirubinemia and pancytopenia, and cholestasis developed. HLH findings were observed in the bone marrow aspiration. Induction chemotherapy as described in the HLH-2004 protocol was initiated. Despite notable improvement in clinical signs and laboratory findings, the infant died, probably due to sepsis, one week after start of chemotherapy HLH should be kept in mind in the differential diagnosis of all cholestatic patients with recently developed cytopenia. For definitive diagnosis of HLH, clinical signs and laboratory findings of the patient should be evaluated, hyperferritinemia and hypertriglyceridemia should be searched and bone marrow aspiration materials should be examined carefully.

Keywords: Hemophagocytic lymphohistiocytosis, Cholestasis, Infant

ÖZET

Kolestazla Başvuran Yenidoan İfantta Hemofagositik Lenfositosis: Olgu Sunumu

Hemofagositik lenfositosis (HLH) nadir ve ölümcül bir hastalıktır. Neonatal kolestaz birçok ciddi yenidoğan hastalığında görülen ortak bir bulgudur. HLH hızla ölümcül olabileceğinden etkili ve hızlı tanı konulması hayati öneme sahiptir. Kliniğimize 10 saatlik, sarılığı olan bir yenidoğan sevk edildi. Laboratuvar tetkiklerinde direkt bilirubinemi, pansitopeni ve kolestaz varlığı saptandı. Yapılan kemik iliği aspirasyonunda HLH bulguları gözlemlendi. HLH-2004 protokolünde belirtildiği şekilde indüksiyon kemoterapisi başlandı. Klinik ve laboratuvar bulgularında görülen iyileşmeye rağmen hastamız kemoterapi başladıktan bir hafta sonra olası sepsis sebebiyle kaybedildi. HLH, sitopeni gelişen tüm kolestatik hastaların ayırıcı tanısında akılda tutulması gereken bir hastalıktır. HLH'nin kesin tanısını koyabilmek için hastanın klinik ve laboratuvar bulguları değerlendirilmeli, hiperferritinemi ve hipertrigliseridemi araştırılmalı ve kemik iliği aspirasyon materyali dikkatlice incelenmelidir.

Anahtar Kelimeler: Hemofagositik lenfositosis, Kolestaz, İfant

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) affects the immune system in both juveniles and adults. It is characterized by the abnormal proliferation of macrophages in various tissues and organs and often results in death. Clinical signs include fever, splenomegaly, icterus, lymphadenopathy, rash, edema, and cerebral dysfunction. In addition to deviations in hemogram variables, impairment in liver functions can also be detected in laboratory analyses.¹ Neonatal HLH with an onset within four weeks after birth is rare, and the diagnosis is frequently delayed.² However, neonatal cholestasis is more common in the newborn period and requires a comprehensive investigation for associated factors. In this article, we present a newborn infant with HLH presenting with manifestations of cholestasis.

CASE REPORT

A 10-hour-old newborn male infant was referred to our clinic for jaundice. He had been born vaginally after 37 weeks of pregnancy. He was the second child of consanguineous parents (second cousins) and his sibling was healthy. His weight, height and head circumference were 2540 g (10-50 p), 47 cm (10-50 p) and 34 cm (50-75 p), respectively. Physical examination was unremarkable except for extensive jaundice and hepatomegaly palpated 2 cm below the costal margin.

Laboratory results on admission were: hemoglobin (Hb) 19.8 g/dl, hematocrit (Hct) 62.3%, reticulocyte 7.5%, platelet count 47,000 mm⁻³, and white blood cell (WBC) count 29,900 mm⁻³ (75% PNL, 25% lymphocyte). Prothrombin time (PT), activated partial thromboplastin time (aPTT) and international normalized ratio (INR) were 16.8 sec, 36 sec and 1.39 (reference limit: 0.8-1.2), respectively. Serum levels of thyroid hormones, α -1 antitrypsin, ammonia, glucose, potassium, urea, and creatinine were within normal ranges. Other results were as follows: aspartate aminotransferase (AST) 391 U/L (35-140), alanine aminotransferase (ALT) 295 U/L (6-50), lactate dehydrogenase (LDH) 180 U/L (170-580), gamma-glutamyl transferase (GGT) 43 U/L (13-147 U/L), alkaline phosphatase (ALP) 98 U/L (28-300), total bilirubin 20.95 mg/dl (< 8), direct bilirubin 10.86 mg/dl (< 0.2), triglyceride 89 mg/dl (36-86), and C-reactive protein (CRP) 1.54 mg/dl (<1). Cerebrospinal fluid examination, sweat test and the test for uri-

nal reductant matter were normal. Serologic tests for TORCH and Parvovirus B₁₉ and blood and urine cultures were all negative.

Viral serologic tests to screen for typical congenital viral infections, brain scans, audiological and ophthalmological examinations, and echocardiogram were evaluated as normal. Abdominal ultrasonography showed normal findings except hepatosplenomegaly. Negative results on tandem mass, urine and blood amino acid chromatography tests excluded the possibility of congenital metabolic diseases. Fever, which was absent at the time of referral, became prominent on day 2. On day 5 of admission, splenomegaly and acholic stool were detected. WBC count, platelet count and Hb decreased to 300 mm⁻³, 9,000 mm⁻³ and 5 g/dl, respectively. Hyperferritinemia (2000 ng/ml) and hypertriglyceridemia (220 ng/dl) developed; however, fibrinogen and albumin levels were within physiological limits. Total bilirubin (25.5 mg/dl), direct bilirubin (16 mg/dl), LDH (601 U/L), GGT (322 U/L), and ALP (418 U/L) increased, consistent with neonatal cholestasis.

Bone marrow aspiration performed on day 20 revealed hemophagocytosis (Figure 1). Upon diagnosis of HLH, the patient was prescribed intravenous administration of gamma globulin (0.4 g/kg/day) for 5 days. There was no improvement in the clinical or laboratory status after gamma globulin therapy. He was administered dexamethasone (10 mg/m²), etoposide (150 mg/m²), cyclosporine (6 mg/kg) and intrathecal chemotherapy, according to the HLH-2004 protocol, on day 27.⁴ On the fourth day of the treatment, total bilirubin and direct bilirubin decreased to 4.7 and 2.2 mg/dl, respectively. Serum AST and ALT levels also decreased to 44 and 49 U/L, respectively. However, WBC count was 200 mm⁻³ and platelet count was 7000 mm⁻³ on the seventh day of HLH treatment. Despite all attempts, the patient died, probably due to sepsis, at the age of 34 days (on the seventh day of chemotherapy). He carried no mutation of the perforin gene.

DISCUSSION

Primary HLH refers to genetic forms, whereas secondary HLH covers acquired forms. The most common form is familial HLH, a primary form, with autosomal recessive inheritance. The parents of a newborn with HLH are likely to be carriers and have sporadic mutations. In 50-80% of the cases, infants up to

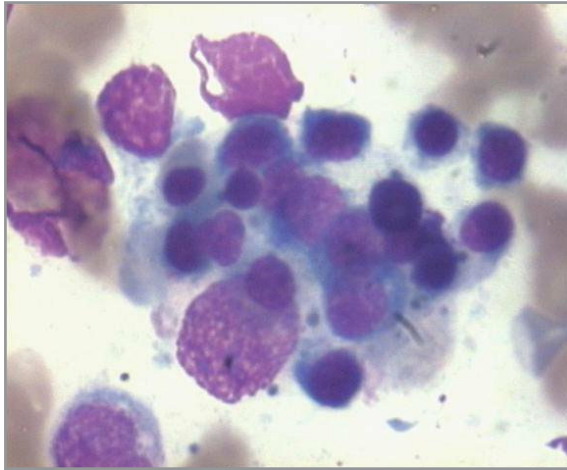


Figure 1. In blood smear, a red blood cell has been seen that had been ingested by a polimorphonuclear cell. H&E, x400

one year of age are affected. Secondary HLH occurs in patients with a suppressed immune system resulting from the boosted activation of immune defense. Viral and bacterial infections, fungal and parasitic infestations, malignant tumors, and intravenous lipid solutions predispose newborns to the secondary HLH.^{1,3} In our patient, HLH was considered the primary form since no other contributing factors could be determined.

The main pathophysiological events in HLH are cytokine dysfunction and generalized histiocytosis resulting from the uncontrollable accumulation of macrophages processing active T-lymphocytes and antibodies.⁵

The criteria for diagnosis of HLH include splenomegaly, cytopenia involving at least two stems, hypertriglyceridemia and hypofibrinogenemia, ferritin ≥ 500 ng/ml, sCD25 ≥ 2400 U/ml, decrease/absence of natural killer cell (NK) activity, and hemophagocytosis without evidence of malignancy in the bone marrow, central nervous system, spleen, or lymph nodes.^{1,4,6} Increased serum transaminases bilirubin, and LDH (>1000 U/L) levels, high protein values in cerebrospinal fluid, the existence of cerebral symptoms accompanied by pleocytosis, hypercytokinemia, generalized coagulation disorders, icterus, temporary eruption, and edema are supportive evidence.¹ All these signs may not be noted at the onset of the disease, but may develop individually or in combination thereafter.^{1,3} The presented case had fever, splenomegaly, pancytopenia, hypertriglyceridemia, and hyper-

ferritinemia as well as hemophagocytosis in the bone marrow aspiration smear. The presence of increased transaminases and evidence of liver dysfunction also supported our diagnosis of HLH.

Perforin 1 (PRF1) gene expression on chromosomes 10q21-22 and 9q21.3-22 has been shown in 20-40% of the HLH cases. The mutation can be familial or sporadic. The existence of nonsense or missense mutations may reflect heterogeneity.^{6,7,8} Factors contributing to the cytolytic dysfunction in HLH include not only the absence of perforin, but also mutations in the Munc13-4 gene, causing the exocytosis of defective granules, and in the syntaxin 11 gene, bridging the relationship between dendritic and NK cells, and other abnormalities as presence of non-functional cytolytic granzymes A and B, transfer of granules, attachment of leukocytes to target membrane, and deficiency in lymphocyte activation.^{1,9}

Neonatal cholestasis, known as jaundice, results from the accumulation of bilirubin, bile acids and cholesterol in the blood and extrahepatic tissues. In addition to persistent icterus during the first two weeks of life, darkened urine, pale or acholic stool and direct bilirubinemia are typical in neonatal cholestasis. Investigation and examination should be comprehensive for the infant with cholestasis because it requires a broad spectrum of evaluations, which should be taken step-by-step, and a handling strategy should be specified for the patient. About 50-70% of the neonatal cholestasis cases are associated with biliary atresia and idiopathic hepatitis in the early stages. However, other factors complicating the case, such as sepsis, nutritional factors causing hepatotoxicity (i.e., galactosemia and tyrosinemia), glycogen storage diseases, endocrine disorders (i.e., hypothyroidism and hypoadrenalism), and infections should be considered primarily and eliminated during the process of diagnosis.^{10,11}

Data on HLH associated with neonatal cholestasis are limited in the literature. Yılmaz et al.¹² performed exchange transfusions twice, in the 31st and 32nd gestational weeks, in a newborn who had ascites and conjugated hyperbilirubinemia at parturition and received blood transfusions twice due to Rh disease and severe anemia. Intravenous immunoglobulin (IVIG, 1 g/kg) was administered upon diagnosis of HLH on day 2. Thrombocytopenia and leukopenia ameliorated on day 6 postpartum. For correction of direct Coombs-positive and symptomatic anemia, the patient received 4 blood transfusions on day 19, and

ferritin level was 5527 ng/ml. Following the elimination of factors that contributed to HLH, on day 28, extramedullary hemopoiesis, cholestasis and iron overloading were confirmed in a liver biopsy. However, no hemochromatosis or cirrhosis was noted. Cholestasis and other signs disappeared in response to deferoxamine administration, as a chelation treatment. As seen in Yılmaz's case, multiorgan failure, autoimmune diseases, congenital hemolytic anemia, blood transfusion, and hypofibrinogenemia may trigger the secondary HLH.¹²

The primary objective in HLH treatment is to suppress hypercytokinemia, which is responsible for the occurrence of devastating symptoms. The HLH-2004 protocol, a standardized treatment, includes the combined use of etoposide cyclosporine A, and corticosteroid (available at URL: www.histio.org/society/protocols). The intravenous administration of IVIG may benefit patients with mild or acquired HLH. Intrathecal treatment is suggested for patients with invaded central nervous system. When the HLH protocol is ineffective, antithymocyte globulin may be suggested. Daclizumab or alemtuzumab administration has also been reported in some cases. Allogeneic bone marrow transplantation is strongly recommended in familial cases, and it should be started as early as possible in bone marrow-suppressed patients.^{1,2,13} In the presented case, in addition to erythrocyte and platelet transfusions, we applied induction chemotherapy as suggested in the HLH-2004 chemotherapy protocol. The patient died on day 7 of the HLH protocol despite significant improvement in some clinical signs and laboratory results. The death probably resulted from sepsis related to severe neutropenia.

In conclusion, HLH is rarely seen in the neonatal period, and abnormal clinical and laboratory findings consistent with the diagnosis of HLH may be seen in many other diseases. Since it is highly fatal, early diagnosis is important for survival. HLH should be considered in the differential diagnosis of cholestasis in a newborn in the presence of additional factors like cytopenia and hyperferritinemia.

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