



Beta Thalassemia Presenting with Neonatal Cholestasis and Extensive Hemosiderosis: A Case Report

Chung Gang Jung, MD, Jang Hoon Lee, MD, PhD and Yu Bin Kim, MD
Department of Pediatrics, Ajou University School of Medicine, Suwon, Korea

ABSTRACT

Neonatal cholestasis is caused by various forms of liver injury and has a complex etiological background. Among these, cases of severe cholestasis due to primary hemolytic disease are rare. Herein, we report a case in which thalassemia-induced severe hemolysis caused bile duct injury by hemosiderosis, with cholestasis occurring shortly after birth and lasting for >4 months. In addition, complete recovery of liver pathology was observed both biochemically and histologically. Hence, clinicians should consider hemolytic disease as a rare cause of neonatal cholestasis in the differential diagnosis of neonates as well as the advisability of conservative treatment based on case progression.

Key Words: Cholestasis; Hemosiderosis; Beta thalassemia; Hemoglobinopathies; Hemolysis

INTRODUCTION

Direct bilirubinemia is defined as an increase in total bilirubin from ≤ 5 to ≥ 1 mg/dL or a direct fraction $\geq 20\%$ if total bilirubin is > 5 mg/dL, which are the accepted standards for clinical cholestasis¹⁾. In neonates, genetic disorders of primary biliary and cellular function and metabolic diseases must be differentiated, and secondary liver injury from various causes must be considered, including abnormal immune responses, hemolysis, hemodynamic changes, and phytosterol accumulation from parenteral nutrition^{2,3)}. In addition, the functionally immature liver of a neonate may respond to changes in systemic status during the perinatal period, causing nonspecific hepatocellular injury and subsequent cholestasis. Cholestasis of unknown etiology is reported in 13% to 26% of newborns⁴⁾. Owing to a complex etiologic background and limited technical resources, diagnostic planning for cholestasis that persists after biliary atresia (BA) differentiation remains a challenging clinical problem.

In cases of persistent cholestasis, the presence of hemolytic disease should be evaluated, as the occurrence of cholestasis and its progression to chronic liver disease due to neonatal hemolytic disease have been demonstrated in some population-based studies⁵⁾. However,

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Correspondence to: Yu Bin Kim, MD
Department of Pediatrics, Ajou
University School of Medicine, 164
World cup-ro, Yeongtong-gu, Suwon
16499, Korea

Tel: +82-32-219-5162

Fax: +82-32-219-5169

E-mail: yubin2107@gmail.com

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the mechanisms of liver pathology that account for this as well as clinical guidelines are unclear. This is attributed to nonspecific hematological and biochemical changes associated with comorbidities in the perinatal circulatory and respiratory systems, making the assessment of specific hemolytic diseases difficult.

Herein, we describe the identification of extensive hemosiderosis due to hemolysis in the course of persistent cholestasis of unknown etiology in a neonate with a history of respiratory circulatory system complications, the diagnosis of β -thalassemia as the underlying cause of persistent cholestasis, and the course of spontaneous recovery of liver pathology.

CASE REPORT

The female patient was born at 38 weeks and 2 days of gestation, with a birth weight of 3,380 g via a repeated elective Cesarean section at another hospital and was transferred to Ajou University Hospital because of respiratory distress immediately after birth. No complications during pregnancy or labor and no significant maternal history were recorded. Both parents are Korean, the patient is the second child, and no specific family history of the disease was reported. Upon arrival at the hospital, her body temperature, blood pressure, heart rate, respiratory rate, and oxygen saturation on room air were 36.5 °C, 64/38 mm Hg, 158 beats/min, 51 breaths/min, and 50%, respectively. The patient was assessed for acute respiratory distress syndrome using chest radiography; hence, a surfactant was administered. Echocardiography was performed for suspected persistent pulmonary arterial hypertension with a >20% difference in upper (right hand) and lower (right foot) oxygen saturation without improvement. The echocardiogram revealed a large 5.3-mm patent ductus arteriosus (PDA), a D-shaped left ventricle, and severe pulmonary hypertension with no improvement in symptoms after the administration of inotropics (dopamine, dobutamine, epinephrine, and norepinephrine), milrinone, and nitric oxide inhalation.

On day 2 of admission, she developed a 39 °C fever, and her condition progressed to sepsis with disseminated intravascular coagulation in blood tests. Subsequently, the patient was administered broad spectrum antibiotics (ampicillin/sulbactam 100 mg/kg and cefotaxime 50 mg/kg twice daily) and immunoglobulin 0.5 mg/kg for 2 days. Her vital signs began to stabilize on

day 6 of admission. PDA ligation was performed on the 21st day of hospitalization, and the patient's respiration stabilized postoperatively. Thereafter, the patient's saturation remained stable without requiring additional oxygenation.

Direct bilirubinemia (6.7 mg/dL) was first detected on day 4 of hospitalization, presumably because of hemodynamic instability due to cardiorespiratory compromise and prolonged total parenteral nutrition. However, direct bilirubinemia (11.9 mg/dL) persisted despite improvement in the respiratory status. Abdominal sonography performed to evaluate the persistent cholestasis revealed a negative triangular cord sign, no apparent findings in the gallbladder (GB), and no findings suggestive of inspissated bile syndrome. Conservative care such as early trophic feeding, cyclic total parenteral nutrition, and other conservative measures were taken; however, direct bilirubinemia (14.7 mg/dL) persisted despite stabilization of the patient's enteral feeding regimen at approximately 24 days of age.

The color of the patient's fecal matter also changed to pale yellow. A hepatobiliary scan was also performed, which indicated decreased hepatic extraction and delayed parenchymal transit times. The GB and intestines were not visualized until 24 hours, and suspected BA was confirmed. Liver biopsy was performed to confirm the diagnosis. Mild portal inflammation with moderate lobular activity was observed with multinucleated hepatocytes, hepatocellular swelling, and severe cholestasis with a canalicular pattern. The initial biopsy confirmed a high probability of neonatal hepatitis. Considering the patient's relatively low gamma-glutamyl transferase levels with a rather nonspecific neonatal hepatitis impression, a gene test (*ABCB11* or *AT8B1*) was performed to rule out progressive familial intrahepatic cholestasis.

Blood transfusions were administered following our neonatal practice guidelines, with a total of eight transfusions provided to sustain a hematocrit (Hct) level between 50% and 55% for infants diagnosed with persistent pulmonary hypertension of the newborn.

From approximately 2 months after the first liver biopsy, a second liver biopsy was performed, which revealed persistent cholestasis with giant cell transformation and diffuse hemosiderin deposition (Figure 1). Magnetic resonance imaging (MRI) and buccal biopsy were performed because of persistently high ferritin levels and liver biopsy findings, despite the typical clinical course of gestational alloimmune liver disease (GALD). Although the MRI confirmed secondary hemochromatosis, the buccal biopsy result was negative.

Blood transfusions were administered according to the established neonatal practice guidelines. These transfusions were predicted based on the necessity for moderate mechanical ventilation, characterized by parameters such as a mean airway pressure (MAP) exceeding 8 cmH₂O for conventional mechanical ventilation (CMV), a MAP of >14 cmH₂O for high-frequency oscillatory ventilation (HFO), or a fraction of inspired oxygen (FiO₂) of >0.4, concomitant with an Hct of <30% and hemoglobin (Hb) level of <10 g/dL. In instances where minimal mechanical ventilation was indicated, interventions such as continuous positive airway pressure, CMV with a MAP of <8 cmH₂O, or HFO with a MAP of <14 cmH₂O, or FiO₂ <0.4, and transfusions were considered if the Hct decreased to <25% and Hb was <8 g/dL. Moreover, when Hct decreased to <20% and Hb decreased to <7 g/dL, an oxygen therapy regimen was implemented exclusively, precluding the need for mechanical ventilation. This approach was similarly applicable in cases of sustained tachycardia or tachypnea persisting >24 hours or a two-fold elevation in oxygen requisites within a 48-hour timeframe, instances of metabolic

acidosis, or significant surgical interventions. When Hct and Hb decreased to <18% and <6 g/dL, respectively, blood transfusion was performed without conditional constraints.

Concurrently, karyotyping was normal (46, XX), a microarray revealed a 2.7 Mb deletion at 11p15.4, which was identified as a β -globin gene cluster including the HBB, HBD, HBE1, HBG1, and HBG2 genes. These genes are associated with heart disease and Hb genes and red blood cell abnormalities⁶). Therefore, assuming that hemolytic cholestasis was caused by hemoglobinopathy, the 18 thalassemia battery for thalassemia, β -thalassemia, and HBB gene confirmation tests were performed, thus subsequently confirming β -thalassemia.

The patient receiving no special medications demonstrated improvement in bile acid-dependent laboratory tests, with a normal range of direct bilirubin levels at 23 weeks of age (Figure 2). Transaminitis also improved. Since then, the patient has had a stable course with normal growth and development, with only folic acid supplementation and without requiring transfusions. A third liver biopsy performed at approximately 1 year of age

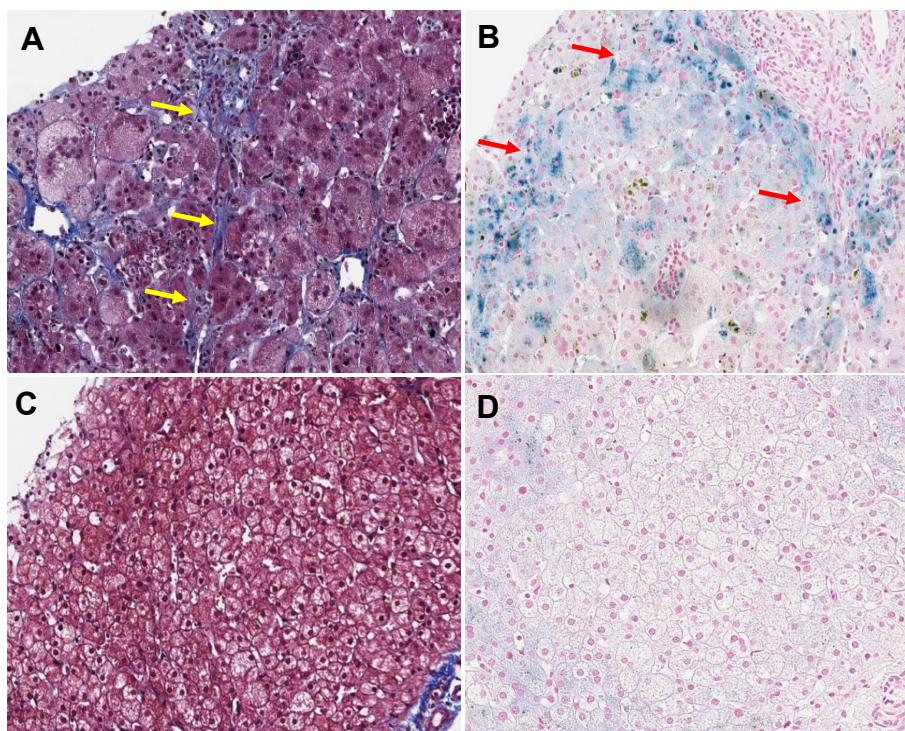


Figure 1. Results of the second liver biopsy and the liver biopsy at the 1-year follow-up. (A) The second biopsy displayed evidence of multifocal extramedullary hematopoiesis and septal and perisinusoidal fibrosis (yellow arrows), indicating neonatal hemochromatosis. These findings correspond with Masson trichrome staining ($\times 200$). (B) The second biopsy exhibited diffuse deposition of hemosiderin within hepatocytes (red arrows), consistent with iron staining ($\times 200$). The biopsy conducted 1 year later demonstrated improvements in both (C) Masson trichrome staining and (D) iron staining (C, D: $\times 200$).

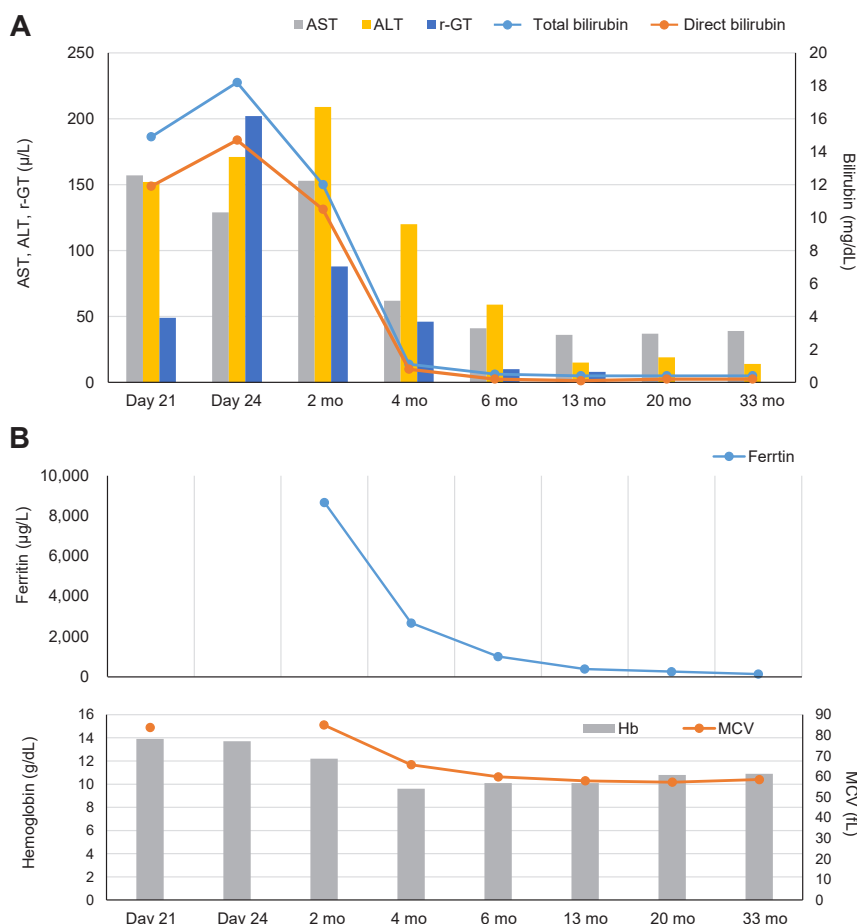


Figure 2. (A) Temporal changes in biochemical laboratory markers of cholestasis. (B) Temporal changes in hematologic markers of hemoglobinopathy and hemolysis. Abbreviations: AST, aspartate transaminase; ALT, alanine transaminase; r-GT, gamma-glutamyl transferase; MCV, mean corpuscular volume.

revealed an intact lobular architecture with multifocal swollen hepatocytes and mild hemosiderin deposition in the periportal areas (Figure 1), confirming overall improvement. Outpatient follow-up indicated stable blood work results.

DISCUSSION

β -Thalassemia is a genetic disorder characterized by reduced or defective synthesis of the β -globin chain in the Hb. It is caused by a point mutation or, more rarely, a deletion in the β -globin gene on chromosome 11, resulting in reduced (β^+) or absent (β^0) synthesis of the β -chain of Hb. This results in inefficient red blood cell production owing to an imbalance in the alpha-globin chain. The clinical presentation is broadly divided into severe, moderate, and mild thalassemia, with individual differences ranging from severe to asymptomatic anemia. The total annual

incidence of symptomatic individuals is approximately 1 in 100,000 people worldwide and 1 in 10,000 people in the European Union. In Korea, the incidence rate is 0.41 in 100,000 people and is gradually increasing owing to the impact of increasing immigration^{7,8)}. A single institutional report from Korea has identified nine pediatric cases over an 11-year period, of which eight were β -thalassemia besides one alpha thalassemia. The median age at diagnosis is 4.3 years, and 78% of cases have an asymptomatic, mild phenotype⁹⁾. In newborns, it can be detected early in life as Coombs-negative hemolytic anemia with hepatosplenomegaly and direct or indirect hyperbilirubinemia due to hemolysis¹⁰⁾.

Thalassemia is diagnosed based on hematological and molecular tests. Microarrays have been reported to have screening utility for patients with thalassemia in Asia¹¹⁾. In the present case, the patient received a definitive diagnosis of the DNA genotype after identification of a large deletion by microarray. Hence,

microarrays may be a safe and rapid screening evaluation tool for primary inherited erythrocyte diseases, including β -thalassemia, in the early stages of a complex clinical situation.

Neonatal hemolysis is classified as an extracellular (acquired) or intrinsic (genetic) red blood cell defect. An overload of iron components due to hemolysis can cause hemosiderosis in the tissues. The severity of cholestasis due to primary erythrocyte disease (hemoglobinopathy) can be observed in sickle cell hepatopathy (SCH), which results in vascular obstructive disorders with hemosiderosis and cholelithiasis of the liver tissue due to intravascular iron overload¹²⁾. However, reports on neonatal cases and natural history are scarce¹³⁾. The histological characteristics of SCH are characterized by Cooper cell erythrocytosis, hemosiderosis, sinusoidal dilatation, and intra-sinusoidal sickling¹⁴⁾. In this case, liver pathology suggested hemosiderin accumulation (siderosis) on two examinations, progression of extramedullary hematopoiesis, perisinusoidal fibrosis, and giant cell formation. It was differentiated from SCH as it was not characterized as a vaso-occlusive disorder but rather exhibited characteristics closer to the pathology of GALD; however, typical GALD levels of necrosis were not observed¹⁵⁾.

GALD has a high fatality rate and requires aggressive intravenous immunoglobulin (IVIG) and plasmapheresis in preparation for liver transplantation and as bridge therapy. Considering the opportunity cost to the patient and burden on technical resources, an urgent exclusionary diagnosis is essential. The persistence of hyperferritinemia on biochemical testing required differentiation from GALD, as did the similarities identified in the histological findings. Although GALD can develop in the early perinatal period and this case was outside the typical timing of GALD onset, we excluded the possibility that the IVIG administration for treating sepsis early in hospitalization may have caused a partial atypical response. Ultimately, the less acute progression of the liver dysfunction, buccal biopsy results, and MRI findings confirmed the exclusion of this diagnosis. The reported positive rate of buccal salivary biopsy in GALD by Smith et al.¹⁶⁾ was 85.7%; however, the biopsy in this case was negative. MRI confirmed the difference in primary/secondary iron deposition characteristics with and without pancreatic sparing, which accords with the conclusions of a previous imaging report¹⁷⁾.

The reversibility of advanced cirrhotic liver changes is difficult to predict in the early stages. Outpatient follow-up and a 1-year biopsy demonstrated a level of biochemical and histological recovery that did not require separate chronic liver disease

management beyond the management of primary hemoglobinopathy. Mechanistic explanations of cholestasis in hemolytic diseases and its subsequent progression to cirrhosis are lacking. Teng et al.⁵⁾ have reported that in a population-based survey in Northern Europe, the rate of cholestasis in patients with neonatal hemolytic anemia was 7% (11/149) and did not progress to chronic liver disease at 2 years of age. In addition, a study on adult transfusion-dependent patients with β -thalassemia has reported that >70% of patients had cirrhotic liver changes of stage 3 or higher, and the extent of this fibrosis was related to age¹⁸⁾. A good natural history may be expected in cases of hemolytic cholestasis and that an early and appropriate diagnosis is important for establishing conservative strategies to minimize iron overload.

To the best of our knowledge, this is the first case report in Korea to evaluate the possibility of progression to cholestasis and cirrhosis due to the hemolytic burden caused by early postnatal hemoglobinopathy. We hope that this case report would encourage further research on the necessity and feasibility of differential diagnosis of hemoglobinopathy-induced hemolysis as a secondary evaluation in patients with neonatal cholestasis.

ARTICLE INFORMATION

Ethical statement

The Institutional Review Board of Ajou University Hospital approved this study (IRB number EX-2023-353). The study was performed in accordance with the Declaration of Helsinki and informed consent for the report was waived by the review board.

Conflicts of interest

Jang Hoon Lee is an editorial board member of the journal, but he was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

Author contributions

Conception or design: Y.B.K.

Acquisition, analysis, or interpretation of data: C.G.J.

Drafting the work or revising: C.G.J., J.H.L., Y.B.K.

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ORCID

Chung Gang Jung <https://orcid.org/0009-0009-3348-6813>

Jang Hoon Lee <https://orcid.org/0000-0003-4765-9948>

Yu Bin Kim <https://orcid.org/0000-0001-6325-6191>

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REFERENCES

1. Fawaz R, Baumann U, Ekong U, Fischler B, Hadzic N, Mack CL, et al. Guideline for the evaluation of cholestatic jaundice in infants: joint recommendations of the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2017;64:154-68.
2. Lane E, Murray KF. Neonatal cholestasis. *Pediatr Clin North Am* 2017;64:621-39.
3. Gotze T, Blessing H, Grillhosl C, Gerner P, Hoerning A. Neonatal cholestasis: differential diagnoses, current diagnostic procedures, and treatment. *Front Pediatr* 2015;3:43.
4. Gottesman LE, Del Vecchio MT, Aronoff SC. Etiologies of conjugated hyperbilirubinemia in infancy: a systematic review of 1692 subjects. *BMC Pediatr* 2015;15:192.
5. Teng J, Wickman L, Reilly M, Nemeth A, Fischler B, Bohlin K, et al. Population-based incidence and risk factors for cholestasis in hemolytic disease of the fetus and newborn. *J Perinatol* 2022;42:702-7.
6. Cao A, Galanello R. Beta-thalassemia. *Genet Med* 2010;12:61-76.
7. Lee HJ, Shin KH, Kim HH, Yang EJ, Park KH, Kim MJ, et al. Increased prevalence of thalassemia in young people in Korea: impact of increasing immigration. *Ann Lab Med* 2019;39:133-40.
8. Lee JS, Rhee TM, Jeon K, Cho Y, Lee SW, Han KD, et al. Epidemiologic trends of thalassemia, 2006-2018: a nationwide population-based study. *J Clin Med* 2022;11:2289.
9. Hong CR, Kang HJ, Lee JW, Kim H, Kim NH, Park KD, et al. Clinical characteristics of pediatric thalassemia in Korea: a single institute experience. *J Korean Med Sci* 2013;28:1645-9.
10. Bender MA, Hulihan M, Dorley MC, Aguinaga MDP, Ojodu J, Yusuf C. Newborn screening practices for beta-thalassemia in the United States. *Int J Neonatal Screen* 2021;7:83.
11. Qin LQ, Yan TZ, Luo SQ, Cai PF, Chen LZ, Zhong QY, et al. Application of DNA microarray in genetic mutation detection in patients with thalassemia. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2021;29:1561-5.
12. Rizvi SA, Ge L, Li S, Pandey A. Acute liver failure in a sickle cell patient. *J Pediatr Gastroenterol Nutr* 2021;72:e54-5.
13. Kotch C, Friedman DF, Wilkins BJ, Samelson-Jones BJ. Conservative management of alloimmune hemolysis and cholestasis with extreme laboratory abnormalities. *Pediatrics* 2021;147:e20193367.
14. Saeed O, Panarelli N, Umrau K, Lee H, Westerhoff M, Cheng J, et al. The histopathologic features of sickle cell hepatopathy: a multi-institutional study. *Am J Clin Pathol* 2022;157:73-81.
15. Silver MM, Valberg LS, Cutz E, Lines LD, Phillips MJ. Hepatic morphology and iron quantitation in perinatal hemochromatosis: comparison with a large perinatal control population, including cases with chronic liver disease. *Am J Pathol* 1993;143:1312-25.
16. Smith SR, Shneider BL, Magid M, Martin G, Rothschild M. Minor salivary gland biopsy in neonatal hemochromatosis. *Arch Otolaryngol Head Neck Surg* 2004;130:760-3.
17. Chavhan GB, Kamath BM, Siddiqui I, Tomlinson C. Magnetic resonance imaging of neonatal hemochromatosis. *Pediatr Radiol* 2022;52:334-9.
18. Kountouras D, Tsagarakis NJ, Fatourou E, Dalagiorgos E, Chrysanthos N, Berdoussi H, et al. Liver disease in adult transfusion-dependent beta-thalassaemic patients: investigating the role of iron overload and chronic HCV infection. *Liver Int* 2013;33:420-7.