Clin. Lab. 2020;66:XXX-XXX

CASE REPORT

Unexplained Late-Onset Hemolytic Jaundice Preceded by High Fetal Hemoglobin Level in an Extremely Low Birth Weight Infant

GoEun Kwon [1, *], Aya Okahashi [1, *], Nobuhiko Nagano [1], Chihiro Nozaki [1], Erina Nakahara [1], Kazumasa Fuwa [1], Hiroshi Yagasaki [1], Hitoshi Kanno [2], Ichiro Morioka [1]

** These authors contributed equally*
[1] Department of Pediatrics and Child Health, Nihon University School of Medicine, Tokyo, Japan
[2] Department of Transfusion Medicine and Cell Processing, Tokyo Women's Medical University, Tokyo, Japan

SUMMARY

Background: **Preterm infants sometimes have transient late-onset hemolytic jaundice; however, the etiology has yet to be determined.**
Case presentation: **In our case, fetal hemoglobin (HbF) level increased significantly to 100% at 23 days of age. Levels of methemoglobin and carboxyhemoglobin also increased to 2.9% and 3.5%, respectively, following the elevated HbF level. At 26 days, hemolytic jaundice developed. No abnormality of red blood cell membranes and enzyme activities was found.**
Conclusions: **The etiology of late-onset hemolytic jaundice in preterm infants may associate with an impaired switching from HbF to adult hemoglobin (HbA) or reverse switching from HbA to HbF.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.190625)

Correspondence:
Nobuhiko Nagano, MD, PhD
Department of Pediatrics and Child Health
Nihon University School of Medicine
30-1 Oyaguchi, Kami-Cho
Itabashi-ku, Tokyo 173-8610
Japan
Phone: +81 3-3972-8111
Fax: +81 3-3957-6186
Email: nagano.nobuhiko@nihon-u.ac.jp

Case Report accepted June 23, 2019

KEY WORDS

late-onset hemolytic jaundice, fetal hemoglobin, extremely low birth weight infant, fetal to adult hemoglobin switching

INTRODUCTION

The causes of hemolytic jaundice in the neonatal period include hereditary spherocytosis, glucose-6-phosphate dehydrogenase deficiency, hemoglobinopathy, blood group mismatch, drugs (i.e., nitrobenzene, vitamin K), infection, hypersplenism, and unknown etiology [1-3]. It has been reported that extremely and very low birth weight infants have transient late-onset hemolytic jaundice with unknown etiology [4-6]. This disease is characterized by decreased arterial oxygen saturation (SpO_2) on pulse oximetry that cannot be attributed to a respiratory condition, and the hemoglobin-related analyses show an increase in methemoglobin (MetHb) level and a secondary increase in carboxyhemoglobin (COHb), with a negative direct Coombs test [5]. May-Grunwald

Giemsa staining shows Heinz bodies [4]. The exact cause of this disease has yet to be determined [4-6]. We encountered a characteristic change in fetal hemoglobin (HbF) level in an extremely low birth weight (ELBW) infant with transient late-onset hemolytic jaundice. HbF to adult hemoglobin (HbA) switching in preterm infants has been reported to be affected by gestational age [7]. HbF level is typically about 70 - 80% at a gestational age or corrected age of 27 weeks [8]. In this case, HbF level was high at 80 to 90% at birth, and further increased to 100% prior to the hemolytic crisis. Here we describe this unusual case of late-onset hemolytic jaundice, preceded by high HbF level in an ELBW infant.

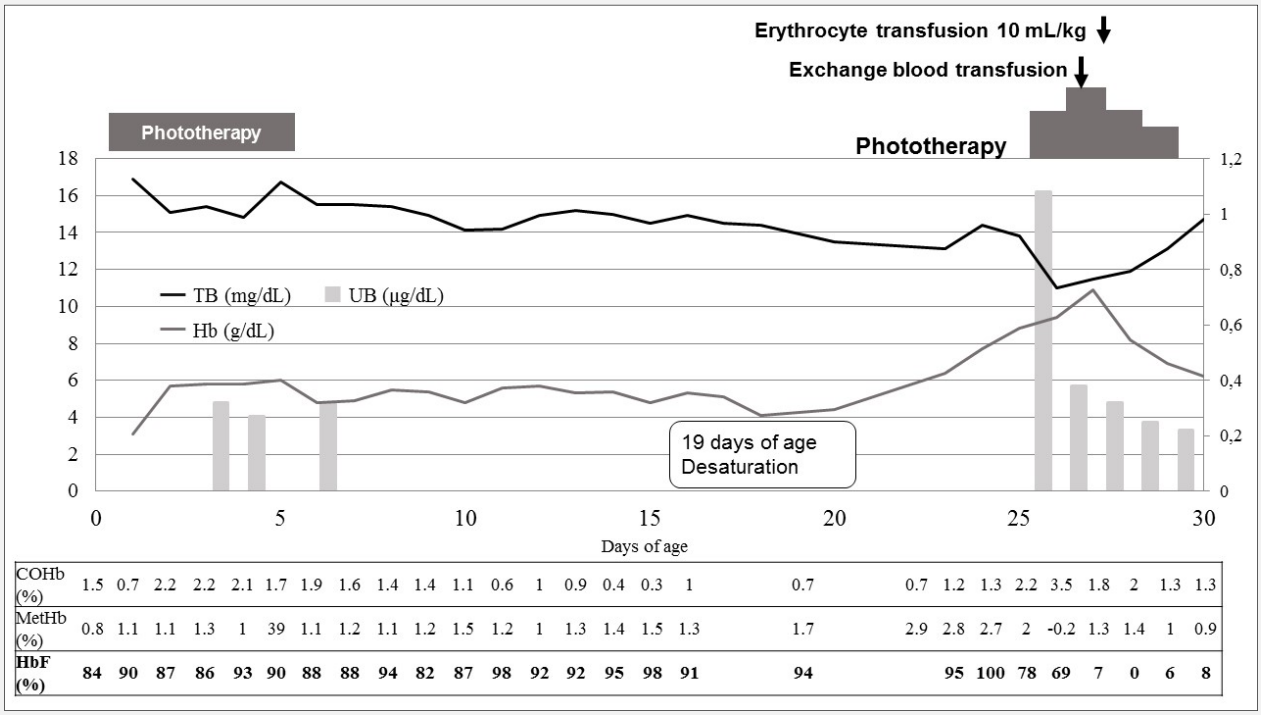
CASE REPORT

The patient was a male infant born via emergency cesarean section at 24 weeks 4 days of gestation (birth weight 671 g [appropriate-for-gestational age], Apgar score 1 and 4 at 1 and 5 minutes, respectively) from a 31-year-old woman (gravida 2, para 1). The mother's blood type was type O Rh-positive, and she was negative for irregular antibodies. There was no known family history of blood disorders. She was hospitalized for complete rupture of membranes at 24 weeks and 1 day of gestations. At delivery, the infant was immediately intubated for respiratory distress syndrome and was admitted to the neonatal intensive care unit. Management was instituted as per routine care for ELBW infants with regard to respiration, circulation, and nutrition. For jaundice, phototherapy was performed from 1 to 5 days of age, after which total bilirubin (TB) remained at about 5 mg/dL, and hemoglobin was maintained through the acute phase at about 13 - 14 g/dL without blood transfusion. HbF level was around 80% after birth and rose to 90% from 10 days of age. Pulse SpO_2 decreased and inspired oxygen fraction was beginning at 19 days of age. Vital signs and basic parameters were normal including body weight 744 g, heart rate 140 - 150 bpm/min, blood pressure 50/28 mmHg, and urine volume 2.2 g/kg/h; no abnormal physical findings were noted. Chest radiographs raised suspicion of bronchopulmonary dysplasia but with no exacerbation and no cardiac enlargement. Therefore, no findings suggesting late circulatory collapse were seen. The hemoglobin-related parameters, such as MetHb, COHb, and HbF, were performed by a blood gas analysis (ABL800 FLEX, Radiometer, Brønshøj, Denmark). MetHb increased at 22 days of age, and HbF significantly increased to 100% at 23 days of age. Subsequently, COHb rose and Hb dropped sharply at 25 days of age. Laboratory examination at 26 days of age showed TB 14.8 mg/dL, Hb 11.7 g/dL, reticulocyte count 63‰, albumin 3.4 g/dL, and unbound bilirubin (UB) 1.08 µg/dL, which was measured by UB analyzer (UA-2, Arrows, Co, Ltd., Osaka, Japan). There was no maternal-fetal blood type incompatibility, and the direct and indirect Coombs tests were both negative. May-Grunwald Giemsa staining revealed that there were no disrupted red blood cells or spherical RBCs, but new methylene staining revealed 62% Heinz bodies (Figure 2A). Based on these results, we considered that the jaundice was possibly associated with non-immune hemolytic jaundice. Phototherapy was started from 26 days of age, but UB was further elevated in the evening of that same day (1.14 µg/dL). Phototherapy was intensified, and exchange blood transfusion (ET) and red blood cell (RBC) transfusion were performed. The levels of TB and UB rapidly decreased, and phototherapy was stopped at 29 days of age. TB level stabilized after ET, and there was no recurrence of hemolytic crisis (Figure 1). Both auditory brainstem response and head magnetic resonance imaging (MRI) at 40 weeks of corrected gestational age were normal.

No findings of chronic bilirubin encephalopathy were found on head MRI at 11 months of corrected age. At 1 year and 5 months of corrected age, athetosis was not observed, and his overall developmental quotient was 73 (Postural-motor area: 97, Cognitive-adaptive area: 63, Language-social area: 97), which was evaluated using the Kyoto Scale of Psychological Development score.

RBC enzyme activity tests and RBC membrane analysis

RBC enzyme activity test and RBC membrane analysis were performed for this case at 149 days of age (3 months after ET). RBC enzyme activity was measured using hemolysate from the isolated RBC fraction, which was filtered through microcrystalline cellulose pellets. The 2 cuvettes, which contained a mixture of 4 reagents (tris-HCL EDTA buffer, $MgCL_2$, NADP, and hemolysate) including water with or without substrate, were scanned at a wavelength of 340 nm. Enzymatic activity in the hemolysate was determined from the initial rate of change of substrate accumulation [9]. The reduced glutathione assay is typically performed via a quantitative reduction reaction by adding 5,5'-dithiobis (2-nitrobenzoic acid) to a compound that has a sulfhydryl group, and the resulting yellow color is detected as absorption at 412 nm [10]. Eosin-5-maleimide (EMA) binding test is a form of flow cytometry generally used to detect RBC membrane defects, such as hereditary spherocytosis. EMA is a fluorescent dye that incorporates into RBC transmembrane proteins and binds to the Lys430 residue in the extracellular loop of band 3. EMA detects decreases in the number of band 3 molecules per RBC as the RBC surface area decreases in hereditary spherocytosis [11]. The EMA binding test revealed no decrease in RBC enzyme activity. No abnormality of RBC membranes and enzyme activities was found in this case (Figure 2B). Finally, this case was diagnosed with unexplained late-onset hemolytic jaundice.



COHb (%)	1.5	0.7	2.2	2.2	2.1	1.7	1.9	1.6	1.4	1.4	1.1	0.6	1	0.9	0.4	0.3	1	0.7	0.7	1.2	1.3	2.2	3.5	1.8	2	1.3	1.3
MetHb (%)	0.8	1.1	1.1	1.3	1	39	1.1	1.2	1.1	1.2	1.5	1.2	1	1.3	1.4	1.5	1.3	1.7	2.9	2.8	2.7	2	-0.2	1.3	1.4	1	0.9
HbF (%)	**84**	**90**	**87**	**86**	**93**	**90**	**88**	**88**	**94**	**82**	**87**	**98**	**92**	**92**	**95**	**98**	**91**	**94**	**95**	**100**	**78**	**69**	**7**	**0**	**6**	**8**	

Figure 1. Clinical course.

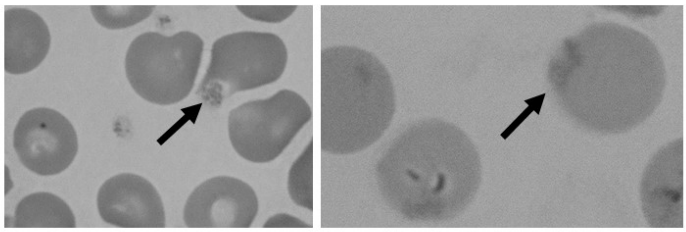
Phototherapy was started beginning at 26 days of age, but unbound bilirubin (UB) was further elevated in the evening of the same day (1.14 μg/dL). Intensified phototherapy was then performed, with exchange blood transfusion and red blood cell (RBC) transfusion. Total bilirubin (TB) and UB levels rapidly reduced, and phototherapy was stopped at 29 days of age.

DISCUSSION

Switching from HbF to HbA is dependent on gestational age rather than time of parturition [7]. In the early stage of pregnancy, ε-globin is strongly expressed in the primitive lineage of yolk sac-derived RBCs, and then via the generation of enucleated blastodermal RBCs from stem cells and progenitor cells in the fetal liver, ε-globin becomes γ-globin. The γ-globin chain combines with the adult α-globin chain to form a stable tetramer to eventually form HbF. Conversion of γ-globin to β-globin takes place in erythroid progenitor cells, converting HbF to HbA [12]. Regarding this conversion, recent studies have revealed the association of the transcriptional repressor BCL11A, which suppresses expression of the fetal globin gene in adults [13]. In this case, HbF tended to increase or not to decrease after birth, and so we considered the possibility of impaired switching from HbF to HbA or reverse switching from HbA to HbF.

HbF levels can be measured by the Kleihauer-Betke test, flow cytometry, high-performance liquid chromatography (HPLC), electrophoresis, and blood gas analysis. In this case, we measured HbF levels by blood gas analysis using the ABL800 FLEX blood gas analyzer. It has been reported that the accuracy of HbF levels measured by blood gas analysis with the ABL 800FLEX is similar to that of HPLC when HbF level was more than 20%. In our case, HbF level was always more than 20%; therefore, we considered that there were few errors in HbF measured by the ABL800 FLEX. Also, Kondo et al. reported the correlation between HbF levels measured with the ABL700 FLEX, with same principle as ABL800 FLEX, and those measured with HPLC. The correlation coefficient is satisfied when SpO_2 is more than 90%, although HbF level is definitely low at levels below 90% of SpO_2. However, our case showed high HbF levels despite low SpO_2. Thus, we concluded HbF level in our case was abnormally high. In addition, increased HbF level preceded the increased levels of MetHb and COHb. It is also likely that HbF may have played a role in the pathogenesis of hemolytic crisis in this disease. However, HbF levels were not monitored in the long-term in other reported cases of ELBW infants with this disease [5]. Further studies should necessarily investigate long-term HbF levels in

A



May-Giemsa staining New methylene staining

B

		Reference value (mean ± 2SD)	Normal Control	This Case
Red blood cell enzyme activity test	Pyruvate kinase (PK), IU/gHb	13.7 - 21.0	16.6	21.3
	Glucose phosphate isomerase (GPI), IU/gHb	55.7 - 73.2	70.5	117
	Glucose 6-phosphate dehydrogenase (G6PD), IU/gHb	6.20 - 9.70	8.81	14.6
	6-phosophogluconate dehydrogenase (6PGD), IU/gHb	7.58 - 11.3	8.73	10.6
	Pyrimidine 5'-nucleotidase (P5N), μ mole P liberated/gHb	8.71 - 12.9	10.4	11.4
Evaluation of glutathione biosynthesis and reduction system	Reduced glutathione (GSH), mg/dL RBC	62.4 - 98.9	88.7	108
Membrane related test	Flow-cytometric osmotic fragility (FCM-OF), %RRC (residual red cells)	52.8 - 88.3	68.6	81.2
	Eosin-5-maleimide (EMA), MCF (mean channel fluorescence)	44.6 - 52.9	51.2	46.5

Figure 2A. May-Grunwald Giemsa staining and new methylene staining.

There were no disrupted RBCs or spherical RBCs, but new methylene staining revealed 62% of Heinz bodies at 26 days of age.

Figure 2B. RBC enzyme activity test and membrane analysis.

No decrease in RBC enzyme activity and no membrane abnormality was found at 149 days of age (3 months after exchange blood transfusion).

this disease.
As a limitation, in this case, there was no abnormality noted in RBC enzyme activity testing and membrane analysis, but the tests were carried out during non-hemolytic crisis. It is necessary to consider the measurement of enzyme activity during the crisis in future cases.

CONCLUSION

An ELBW infant with late-onset hemolytic jaundice preceded by high HbF level was reported. In some cases, HbF may tend to increase and abnormally high values may be seen as preceding symptoms. It is necessary to investigate the relationship between this disease and HbF level in order to clarify the causes of this disease.

Acknowledgment:
We thank Drs. Ryoji Aoki, Midori Hijikata, Kazunori Kayama, Ryota Kato, and Ayako Seimiya for their support in the diagnosis and treatment of this patient in the neonatal intensive care unit of Nihon University Itabashi Hospital. We also thank Dr. Takako Aoki (Tokyo Women's Medical University Hospital) for performing the RBC enzyme activity tests and membrane analysis. This research was supported by the Practical Research Project for Intractable Diseases from Japan Agency for Medical Research and Development, AMED (19ek010 9265h0003).

Disclaimers:
None.

Support:
The authors received no financial support for the research, authorship, and/or publication of this article.

Declaration of Interest:
The authors declare that they have no conflicts of interest.

References:

1. Gross RT, Bracci R, Rudolph N, Schroeder E, Kochen JA. Hydrogen peroxide toxicity and detoxification in erythrocytes of newborn infants. Blood 1967;29(4);481-93 (PMID: 6023063).

2. Kirsh IR, Cohen HJ. Heinz body hemolytic anemia from the use of methylene blue in neonates. J Pediatrics 1980;96:276-7 (PMID: 6766185).

3. Vitkun SA, Smith RP, French EE, Edwards WH, Watkins N. Heinz body hemolytic anemia in newborns and failure of laboratory studies to implicate a phenolic disinfectant. Pediatrics 1983; 71:352-8 (PMID: 6828342).

4. Miyazono Y, Hirono A, Miyamoto Y, Miwa S. Erythrocyte enzyme activities in cord blood of extremely low-birth-weight infants. Am J Hematol 1999;62:88-92 (PMID: 10509002).

5. Jinnou H, Sugiura H, Kikuchi S, Shirai K, Hirose E, Ohki S. Unexplained late-onset hemolysis and methemoglobinemia in a preterm infant. Pediatr Int 2011;53(6):1084-7 (PMID: 22181569).

6. Nagasaka M, Kikuma T, Iwatani S, et al. Transcutaneous bilirubin monitoring predicts unexplained late-onset hemolysis in a very low birthweight infant. BMC Res Notes 2016;9:153 (PMID: 26965169).

7. Colombo, B. Kim B, Atencio RP, Molina C, Terrenato L. The pattern of fetal hemoglobin disappearance after birth. Br J Haematol 1976;32:79-87 (PMID: 1259928).

8. Watanabe Y, Osawa K, Sato I, et al. Foetal haemoglobin concentration at postmenstrual age is unaffected by gestational age at birth. Ann Clin Biochem 2018;55(3):400-3 (PMID: 28656818).

9. Hirono A, Kanno H Miwa S, Beutler E. Pyruvate kinase deficiency and other enzymopathies of the erythrocyte. The Online Metabolic and Molecular Bases of Inherited Diseases. McGraw-Hill; 2001:4637-64 (DOI: 10.1036/ommbid.215).

10. Palek J, Sahr KE. Mutations of the red blood cell membrane proteins: from clinical evaluation to detection of the underlying genetic defect. Blood 1992;80:308-30 (PMID: 1627793).

11. King MJ, Smythe JS, Mushens R. Eosin-5-maleimide binding to band 3 and Rh-related proteins forms the basis of a screening test for hereditary spherocytosis. Br J Haematol 2004; 124:106-112 (PMID: 14675415).

12. Schroeder WA, Huisman THJ, Brown AK, et al. Postnatal changes in the chemical heterogeneity of human fetal hemoglobin. Pediatr Res 1971;5:493-9.
https://www.nature.com/articles/pr1971360.pdf

13. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. Cold Spring Harb Perspect Med 2013;1:3(1):a011643 (PMID: 23209159).