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Case Report

Neonatology Section

# Use of Red Cell Indices for Screening of Hereditary Spherocytosis in Neonates: A Report of Two Cases Confirmed by Exome Sequencing

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## ABSTRACT

Prolonged unconjugated hyperbilirubinaemia is defined as neonatal jaundice persisting beyond 14 days of life. Hereditary Spherocytosis (HS) often remains underdiagnosed and is a notable cause of prolonged neonatal jaundice. Coombs negative haemolytic anaemias are an important cause of unconjugated hyperbilirubinaemia, and HS is one of the most common inherited red cell membrane disorders. Other causes of prolonged unconjugated jaundice include extravasated blood (cephalhaematoma, bruises, intraventricular haemorrhage in preterm infants), haemolysis (blood group incompatibility, red blood cell enzymopathies, and membranopathies), urinary tract infection, congenital hypothyroidism, and rare familial disorders such as Gilbert's syndrome and Crigler-Najjar syndromes I and II. Herein, the authors present a case report of two male neonates (three week-old and 25day-old) who presented with hyperbilirubinaemia requiring intensive phototherapy, blood transfusion, an exchange transfusion, and were diagnosed with HS. A significant family history suggested haemolytic anaemia, and red cell indices, namely Mean Corpuscular Haemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), and the ratio of MCHC/MCV (neonatal HS index), helped suspect HS. Whole exome sequencing identified the specific mutation and confirmed the diagnosis of HS. Both of these neonates presented with prolonged neonatal jaundice, and HS was suspected based on the family history and red cell indices, namely MCHC and MCHC/MCV ratio, as highlighted in the present case series.

**Keywords:** Anaemia, Haemolysis, Jaundice, Newborn, Spherocytes

## CASE REPORT

### Case 1

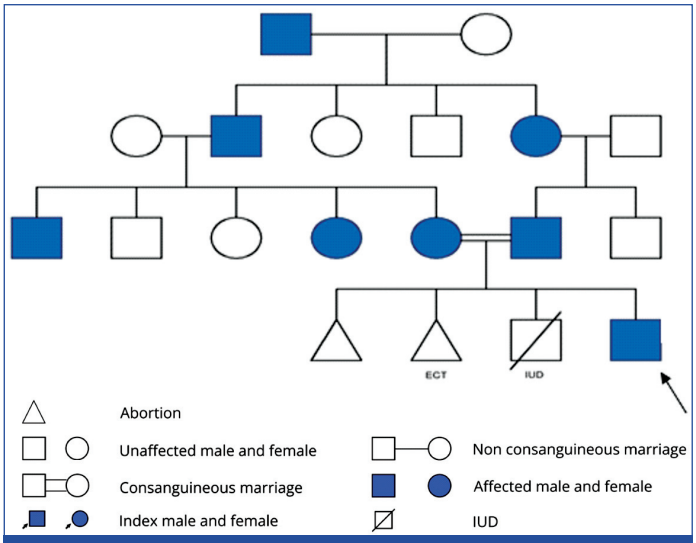
A three-week-old male neonate was referred to the unit for prolonged neonatal jaundice. This baby was born at 36 weeks gestation to a G4A2D1 mother by caesarean section done for foetal distress. The baby was a product of consanguineous marriage. The baby cried immediately after birth and weighed 2950 gm. The mother's blood group was B negative. The Indirect Coombs Test (ICT) was negative. The mother received two doses of anti-D (300 µg), the first dose at 28 weeks and the second one after delivery. The baby's blood group was B positive. At 48 hours of life, the baby developed jaundice with a Total Serum Bilirubin (TSB) of 17.8 mg/dL, was treated with phototherapy for two days, and was discharged when the bilirubin decreased to 10.6 mg/dL. The baby was readmitted on day nine of life with a TSB of 21.8 mg/dL and received phototherapy for another three days.

On day 22 of life, the baby was referred to the hospital for prolonged jaundice. On examination, the baby was icteric and pale without hepatosplenomegaly. Blood tests showed TSB of 21.9 mg/dL (direct fraction: 1.3 mg/dL), Haemoglobin (Hb) of 8.7 gm/dL, normal peripheral smear, and a reticulocyte count of 4.1%. The Direct Coombs Test (DCT) was negative, and the thyroid profile was normal. Red cell indices showed an increased MCHC (39.3 gm/dL) and MCHC/MCV ratio of 0.42, suggestive of HS [Table/Fig-1]. Both parents had a history of jaundice in the neonatal period and received phototherapy. The baby's mother had received blood transfusions twice during her childhood for anaemia (Hb <7 gm/dL). The paternal grandmother had a history of jaundice in infancy (bilirubin 3.3 mg/dL, conjugated bilirubin 0.6 mg/dL) and anaemia during childhood and adolescence (Hb 7.2 gm/dL) and received blood transfusions twice. She also developed cholelithiasis and was advised splenectomy at 15 years of age [Table/Fig-2]. Mother's laboratory tests showed a negative DCT, Lactate Dehydrogenase (LDH) 872 units/L, and haptoglobin <13 mg/dL, suggestive of haemolytic anaemia. Osmotic fragility, sickling, and Hb electrophoresis tests were normal.

| Patient characteristics | Case 1 | Case 2 |
|---|---|---|
| Gestational age (in weeks) | 36 | 37 |
| Birth weight (in grams) | 2950 | 2400 |
| Age (days) at presentation | 22 | 25 |
| Family history | Mother, father, grandmother, uncle | Father |
| Hb at presentation (gm/dL) | 8.7 | 4.5 |
| Bilirubin at presentation (mg/dL) | 21.9 | 9.2 |
| MCHC/MCV | 0.42 | 0.41 |
| MCHC (gm/dL) | 39.3 | 36.9 |
| MCV (fL) | 94.4 | 89.2 |
| Reticulocyte count (%) | 4.1 | 14 |
| Peripheral smear | Normal | Normal |
| Exome sequencing | SLC4A1 mutation affecting band 3 protein | SPTB mutation affecting β-spectrin |
| Treatment received | Phototherapy | Phototherapy, PRBC transfusion |
| Duration of hospital stay (in days) | 4 | 2 |

**[Table/Fig-1]:** Clinical characteristics, investigations, and management.
PRBC: Packed red blood cell

The baby had a normal Glucose-6-phosphate Dehydrogenase (G6PD) assay, normal LDH (350 units/L), and the osmotic fragility test was inconclusive. As the Eosin-5'-maleimide (EMA) binding test was not available, whole exome sequencing was performed. The baby received phototherapy for two days and was discharged on folate supplements (vitcofol drops, 1 mL contains 200 µg of folic acid). Exome sequencing revealed solute Carrier family 4 member 1




**[Table/Fig-2]:** Pedigree chart of case 1 illustrates autosomal dominant mode of inheritance arrow points to the index case.

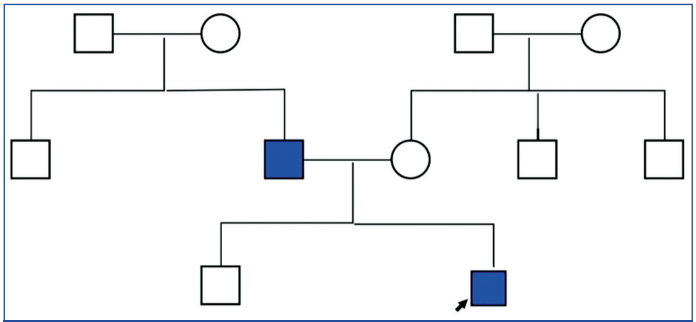
(SLC4A1) mutation encoding the band 3 protein, which confirmed the diagnosis of HS involving the band 3 protein [Table/Fig-3]. At three months of age, the baby had mild jaundice (bilirubin 4.2 mg/dL) and anaemia (Hb 8.9 g/dL), with normal growth and development.

| Gene (transcript) | Location | Variant | Zygosity | Disease (OMIM) | Inheritance |
|---|---|---|---|---|---|
| SPTB NM_001024858.4 | Exon 13 | c.1920G>A (p.Trp640Ter) | Heterozygous | Spherocytosis type 2 (OMIM#616649) | Autosomal dominant |

**[Table/Fig-3]:** Whole exome sequencing report of case 1.
OMIM: Online mendelian inheritance in man

## Case 2

A 25-day-old male neonate was brought to the outpatient department for jaundice. On examination, the baby was icteric, pale, and had mild splenomegaly. He was born at 37 weeks gestation by emergency caesarean section done for preeclampsia. The baby was vigorous at birth and weighed 2400 grams. At 44 hours of life, the baby was noted to be icteric. Laboratory investigations showed a TSB of 18 mg/dL, Hb of 14.2 g/dL, negative DCT, and normal thyroid profile. The blood groups of the mother and baby were O positive. The baby received phototherapy for two days and was discharged when the TSB decreased to 11.0 mg/dL. The baby is a non consanguineous product, and the father had a history of jaundice at 18 years of life (total bilirubin 2.3 mg/dL, direct fraction 0.4 mg/dL) [Table/Fig-4].



**[Table/Fig-4]:** Pedigree chart of case 2. Arrow points to the index case.

On day 25 of life, the baby was referred to the outpatient department for evaluation of jaundice. Laboratory investigations showed a bilirubin level of 9.2 mg/dL (direct fraction: 1.0 mg/dL), Hb of 4.5 g/dL, reticulocytosis (14%), normal peripheral smear, negative osmotic fragility test, and negative DCT suggestive of Coombs negative haemolytic anaemia. Red cell indices showed an elevated MCHC of 36.9 g/dL and an increased HS index (ratio of MCHC/MCV) of 0.41, suggestive of HS [Table/Fig-1]. The baby received packed cell transfusion for anaemia. Whole-exome sequencing showed a mutation in the SPTB gene, which encodes β-spectrin activity, confirming the diagnosis of HS involving β-spectrin protein [Table/Fig-5]. At two months of age, the baby had jaundice (total bilirubin 3.9 mg/dL, direct fraction 0.4 mg/dL) and anaemia (Hb 6.2 g/dL) and again received a packed cell transfusion. The baby's growth and development were normal at five months of age.

| Gene (transcript) | Location | Variant | Zygosity | Disease (OMIM) | Inheritance |
|---|---|---|---|---|---|
| SLC4A1(-) (ENST00000262418.12) | Intron 18 | c.2481+1G>A (5' splice site) | Heterozygous | Spherocytosis type 4 (OMIM#612653) | Autosomal dominant |

**[Table/Fig-5]:** Whole exome sequencing report of case 2.
SLC4A1: Solute Carrier family 4 member 1; OMIM: Online mendelian inheritance in man

## DISCUSSION

The HS is an intrinsic disorder of red cell membrane proteins, namely ankyrin 1, band 3, β-spectrin, α-spectrin, and protein 4.2. It results in spherical and poorly deformable red cells that undergo haemolysis [1]. HS affects individuals of all ethnicities, with a high prevalence of 1 in 2000 to 1 in 5000 in Caucasians and a low prevalence of 1.27 in 100,000 in Chinese and Asians [2,3]. The genetic defects causing HS include mutations in the ANK1 gene encoding ankyrin 1, the SLC4A1 gene encoding band 3 protein, and the SPTB gene encoding β-spectrin. These mutations are inherited dominantly in 75% of cases, while mutations in the SPTA1 gene encoding α-spectrin and the EPB42 gene encoding protein 4.2 are inherited recessively or arise as de novo mutations in 25% of cases [4]. Ankyrin 1 is the most commonly involved membrane protein, followed by band 3 mutations. Most of these mutations result in mild to moderate disease, except for α-spectrin mutations, which cause a severe form of HS [5].

The clinical spectrum of HS ranges from asymptomatic neonates to severe foetal anaemia and hydrops foetalis. Jaundice is the most common presenting symptom, followed by anaemia [6]. In 65% of cases, atleast one parent has a positive history of jaundice and anaemia. A history of anaemia, jaundice, blood transfusions, splenectomy, and gallstones in family members suggests haemolytic anaemia [7]. A positive family history in both of these cases and the identified mutations indicates an autosomal dominant mode of inheritance.

Blood tests usually reveal anaemia with a relatively normal reticulocyte count due to sluggish erythropoietic response in the first few weeks after birth. In one-third of neonatal HS cases, spherocytes are not seen on peripheral smear [8]. In both of these cases, the peripheral smear was normal, but the MCHC and MCHC/MCV ratio were high. Two cases of neonatal HS reported from China presented with hyperbilirubinaemia in the first 24 hours of life, and both had STBP mutations involving β-spectrin [9,10]. Christensen RD et al., reported that an MCHC of >36 g/dL had 82% sensitivity and 98% specificity in diagnosing HS [11]. In the Intermountain healthcare database, an MCHC/MCV ratio of >0.36 had 97% sensitivity, 99% specificity, and >99% negative predictive value for diagnosing HS [12].

The laboratory evaluation of HS includes the incubated osmotic fragility test, cryohaemolysis test, osmotic gradient ektacytometry, EMA binding test, and sodium dodecyl sulfate polyacrylamide gel electrophoresis {Sodium Dodecyl-sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)} test. The SDS PAGE test can identify

the defective membrane protein [13]. The Eosin-5'-maleimide (EMA) dye binds to membrane proteins, and the loss of membrane proteins results in decreased fluorescence intensity of EMA-tagged erythrocytes. Osmotic gradient ektacytometry uses a laser-diffraction viscometer to measure the deformability of the erythrocytes [14].

In both cases, whole exome sequencing was done as none of the affected family members had a definite diagnosis. Parents should be made aware of folate supplementation, blood transfusions, gallstones, and splenectomy in later life. Vaccination against encapsulated microorganisms such as Pneumococci, Haemophilus influenzae type b, and Neisseria meningitidis should be given [15].

## CONCLUSION(S)

The HS is an underdiagnosed cause of prolonged neonatal jaundice. Diagnostic challenges in the neonatal period include inconclusive peripheral smear, absence of spherocytes, lack of reticulocytosis, inconclusive osmotic fragility test, and limited availability of EMA binding assay, Ektacytometry, and SDS-PAGE tests. A positive family history and red cell indices (MCHC, MCHC/MCV ratio) help to suspect HS. Prompt diagnosis, treatment, and parental counseling help to avoid complications and improve the outcome of this disorder.

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