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Disease-modifying influences of coexistent G6PD-deficiency, Gilbert syndrome and deletional alpha thalassemia in hereditary spherocytosis: A report of three cases

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ABSTRACT

Background: Hereditary spherocytosis (HS) is a common inherited hemolytic anemia characterized by heterogeneous clinical presentations with variable degrees of anemia, jaundice, splenomegaly and gallstones. Although the underlying genetic defects in red cell membrane proteins may explain many phenotypic variations, a proportion of variability may be due to other co-inherited factors like enzymopathies, thalassemias and Gilbert syndrome. Associations of HS with glucose-6-phosphate dehydrogenase (G6PD) deficiency and Gilbert syndrome in isolation have been reported previously.
Methods: We describe 3 adult cases of HS with concomitant Gilbert syndrome and G6PD-Mediterranean mutations (2 hemizygous males, aged 15 and 35 y and 1 heterozygous 25-y female).
Results: Two patients required multiple transfusions that required splenectomy for management. One patient (15 y male) also carried the single gene alpha 4.2 deletion and was less symptomatic.
Conclusions: These cases illustrate the importance of clinico-pathological correlation and judicious extended testing for various contributing factors that may modify the clinical course of HS patients. G6PD deficiency is also a common enzymopathy in India and can contribute to the phenotypic heterogeneity. Its recognition is important for advising avoidance of oxidizing drug exposure.

1. Introduction

Hereditary spherocytosis (HS) comprises a group of relatively common inherited hemolytic anemias characterized by spherical, osmotically-fragile erythrocytes on blood films. The basic underlying defects are red cell membrane protein abnormalities that result in impaired deformability. This leads to preferential splenic entrapment and hemolysis of spherocytic red cells [1].

HS can be diagnosed based on clinical manifestations of anemia, jaundice, early gallstones, splenomegaly and a positive family history in many cases corroborated by laboratory findings of spherocytosis, reticulocytosis, hyperbilirubinemia, increased lactate dehydrogenase (LDH), increased incubated osmotic fragility (iOFT) and reduced fluorescence intensity of eosin-5-maleimide (EMA)-labeled red cells on flow cytometry [2]. Patients may show marked clinical heterogeneity that has been typically ascribed to the underlying genetic defects in red cell membrane proteins. However, several reports also exist of concomitant second disorders that may worsen [3,4], or less commonly ameliorate [5] specific components of the HS phenotype. We report three interesting cases where the interaction between HS, glucose-6-dehydrogenase deficiency and the Gilbert syndrome led to a variably severe phenotype. These cases illustrate the clinical and therapeutic importance of making the correct diagnoses in such patients.

2. Case reports

2.1. Case 1

A 35-y-old male agriculturist had been first diagnosed with glucose-6-phosphate dehydrogenase (G6PD) deficiency at the age of 3 y. Since then he reported recurring episodes of jaundice and symptomatic anemia, especially following fever and in summers. He had required >25 blood transfusions in his lifetime despite avoiding oxidant drugs and was intermittently taking hematinic supplements. Family history was positive as his mother too reported recurrent jaundice and occasional

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blood transfusion requirement, although less than that of the index case.

In the current admission to our hospital, the patient presented with severe anemia and mild jaundice following high-grade fever, and possible anti-malarial therapy outside. On examination, he was icteric, pale with the spleen palpable 10 cm and the liver 4 cm below the respective costal margins. Investigations revealed hemoglobin (Hb) — 52 g/l, mean corpuscular volume (MCV) — 100.4 fl, mean corpuscular hemoglobin (MCH) — 34.7 pg, mean corpuscular hemoglobin concentration (MCHC) — 36.6 g/dl, red cell distribution width- coefficient of variation (RDW-CV) — 22.3% and uncorrected reticulocyte count 68% with normal total leukocyte and platelet counts. A blood film revealed anisopoikilocytosis with macrocytes, many microspherocytes and polychromatophilic red cells. His last transfusion was administered 1.5 months back; the microspherocytes were therefore unlikely to be transfusion-related. Total bilirubin was 3.0 mg/dl (conjugated fraction 0.23 mg/dl) with normal liver enzymes and other liver and renal function tests.

G6PD-deficiency was reconfirmed by a positive methemoglobin-reduction (MR) test. Restriction fragment length polymorphism (RFLP) using *MboII* restriction endonuclease revealed hemizygosity for the G6PD Mediterranean mutation [n.563 C>T], WHO Class II variant [6]. Since splenomegaly, microspherocytic red cells and elevated MCHC were anomalous for Mediterranean-type G6PD-deficiency; additional tests for hemolysis were done. Direct Coombs test, hemoglobin high performance liquid chromatography (HPLC) and serum and urine hemoglobin were all normal or negative. Incubated OFT revealed mildly increased red cell sensitivity to osmotic lysis with median corpuscular fragility of 6.0 g/l NaCl (normal range: 4.65–5.9). Flow cytometric EMA dye-binding test revealed mean channel fluorescence (MCF) ratio 0.68 (reference range > 0.80), thus confirming HS. The EMA dye binding test involves gating of red cells on log forward versus log side scatter (top two scatter plots). The reduction in EMA fluorescence in the patient vis-a-vis one of the controls is visualized on the combined overlay histogram (Fig. 1). The specimen was acquired on a Becton Dickinson FACS Canto II flow cytometer (BD Biosciences) and analyzed for this publication using Flowing Software™ v.2.5.1 (courtesy Cell Imaging Core, Turku Centre for Biotechnology). EMA testing of his family revealed MCF ratio of 0.81 in his mother (borderline result, possibly HS) and 0.63 and 0.9 in his son and daughter respectively. His mother was also heterozygous for G6PD deficiency on molecular testing. The autosomal dominant pattern of inheritance in three generations could be demonstrated in this family.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was attempted on purified red cells to identify the defective protein. Protein extracted from RBC ghosts was quantified and loaded on a 10% SDS-polyacrylamide gel. However, no conclusive reduction in any protein could be definitively identified (Fig. 2).

Sanger sequencing of the 5′UTR (untranslated region) of the *UGT1A1* (uridine diphosphate glucuronosyl transferase A1) gene was done. The patient was homozygous for $[TA]_{7/7}$ repeats in the promoter region, confirming Gilbert syndrome. The normal number of repeats is 6/6. Automated DNA sequencing was done on an ABI 3130 Genetic Analyzer (Applied Biosystems) and analyzed using Sequencher® ver 5.4 sequence analysis software, (Gene Codes Corp.). He was managed conservatively and the Hb improved considerably. Splenectomy was done subsequently and the Hb and symptoms have normalized significantly. Presently he is transfusion-independent (Hb >140 g/l) although mild jaundice is persisting.

2.2. Case 2

A 25-y old primigravida presented at term to her local hospital with severe anemia and decreased fetal movements. She was transfused

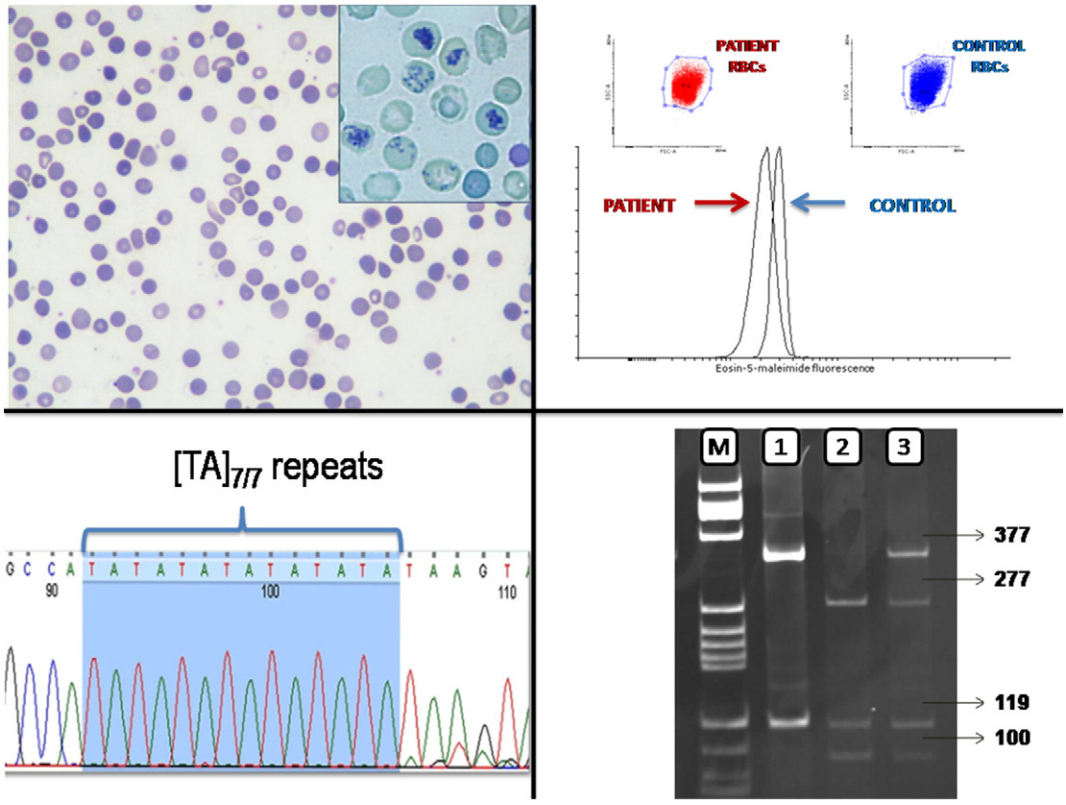

Fig. 1. Panel of investigations performed in our 3 cases. **Top left:** Blood film from Case 1 showing numerous spherocytes (*Leishman stain, original magnification ×1000*). **Inset** shows reticulocytosis (*New methylene blue dye, original magnification ×1000*). **Top right:** The EMA dye binding test. **Bottom left:** Sequencing chromatogram of *UGT1A1* gene with homozygosity for $[TA]_{7/7}$ repeats. **Bottom right:** PCR-RFLP visualized on 2% agarose gel electrophoresis. The lane on the extreme left shows a pBR322 marker. Lane 1: normal for G6PD Mediterranean mutation; lane 2: Hemizygous G6PD Mediterranean mutation; lane 3: heterozygous G6PD Mediterranean mutation.