Case Report

Amalgamation of the Rarest: Homozygous Beta Thalassemia and Gilbert Syndrome

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DOI: 10.21276/APALM.3365

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Submitted: 29-Apr-2024
Final Revision: 16-Jun-2024
Acceptance: 14-Jul-2024
Publication: 11-Aug-2024

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Abstract

Homozygous beta-thalassemia patients are known to show significant variability in serum unconjugated bilirubin levels, which can be attributed to the red cell destruction rate, ineffective erythropoiesis, or bilirubin elimination capacity. One cause of this hyperbilirubinemia is Gilbert syndrome ((TA)7/(TA)7 genotype), which is known to act as a modifying factor in thalassemic patients. Defective glucuronidation in Gilbert allele carriers aggravates jaundice in all hemolytic anemias, including thalassemia. Here, we present a rare case of the amalgamation of thalassemia intermedia and Gilbert syndrome in a young male patient.

Keywords:

Beta-thalassemia, Gilbert syndrome, Hyperbilirubinemia, Thalassemia intermedia

Introduction

Hemoglobin (Hb) disorders confer a significant health problem in 71% of 229 countries [1]. Being one of the most serious and common genetic abnormalities worldwide, thalassemia accounts for 17% of over 330,000 affected infants born annually. It is defined as a heterogeneous autosomal recessive disorder caused by a mutation in the α (HBA1/HBA2) and β globin (HBB) genes. Anything that leads to either quantitatively low production of Hb chains or a qualitatively structural defect in the globin chain will lead to various hemoglobinopathies [2]. In thalassemia, 200 disease-causing mutations have been identified, resulting in marked heterogeneity [3]. Homozygous beta-thalassemia patients, on the other hand, also show significant variability in serum unconjugated bilirubin levels, which can be attributed to the red cell destruction rate, ineffective erythropoiesis, or bilirubin elimination capacity [4]. In the absence of signs of hemolysis, reasons for hyperbilirubinemia caused by either inherited

hematological disorder or any other cause need to be evaluated. It is known that bilirubin is primarily catabolized by the hepatic enzyme uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1), which mediates the conjugation of bilirubin to a water-soluble form [5]. Reduced activity of this enzyme, due to an additional dinucleotide resulting in a (TA)7 motif, results in a benign form of hyperbilirubinemia, i.e., Gilbert syndrome, which can lead to high levels of bilirubin and a 30% reduction in UGT1A1 gene expression. Various diseases like sickle cell disease, thalassemia, and glucose-6-phosphate dehydrogenase deficiency are known to have raised bilirubin levels when co-inherited with Gilbert syndrome [4]. Defective glucuronidation in Gilbert allele carriers aggravates jaundice in all hemolytic anemias, including thalassemia. Here, we present a rare case of a young patient with a thalassemia intermedia phenotype along with a rare association of a disorder affecting serum bilirubin levels, most likely Gilbert syndrome.

Case Report

A 22-year-old male presented to our hospital with chief complaints of severe icterus, pallor, and splenomegaly. He was diagnosed with homozygous beta-thalassemia at 10 years of age at a referral institute, with a thalassemia intermedia phenotype. There was no history of past blood transfusions, although there was a positive history of a few episodes of increasing pallor and icterus.

His initial investigations, including a complete blood count, revealed reduced Hb (7 g/dL), reduced red cell indices, and a raised reticulocyte count (4.5%). His liver function tests were also deranged, with a total bilirubin level of 4.3 mg/dL and raised serum ferritin of 200.3 ng/mL. The peripheral blood film revealed a dimorphic RBC population, including microcytic hypochromic and macrocytic cells, with severe anisopoikilocytosis in the form of teardrop and target cells (Figure 1). He was further given vitamin B12 and folic acid therapy and kept on monthly follow-ups with repeated blood investigations, which were later compared with pre-supplementation therapy levels (Table 1).

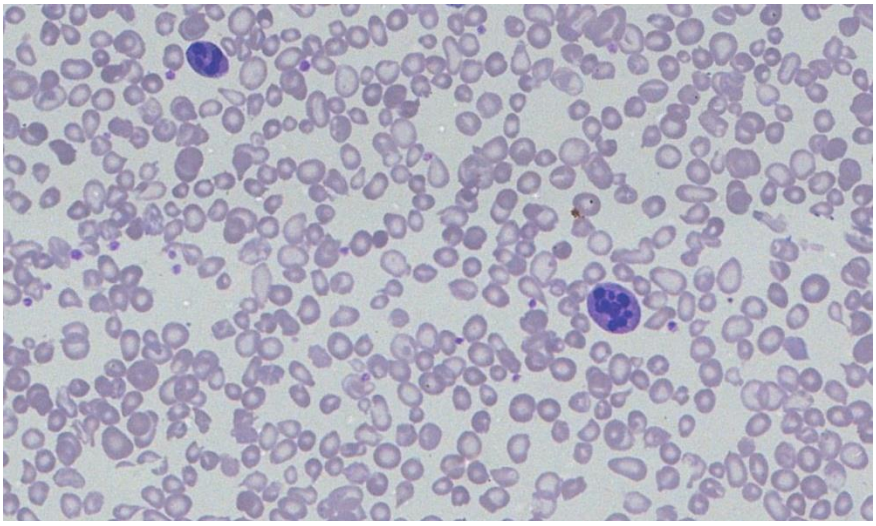

Figure 1: Peripheral blood film shows dimorphic RBC morphology with severe anisopoikilocytosis (Leishman, x100).

High-performance liquid chromatography (HPLC) revealed elevated HbA2 (6%) and HbF (26.70%), leading to a diagnosis of beta-thalassemia intermedia (Figure 2). Following treatment, Hb showed an improving trend, but bilirubin remained disproportionately high (7.5 mg/dL) for the degree of anemia and hemolysis. Further evaluation for this increased unconjugated hyperbilirubinemia was done by UGT1A1 gene polymorphism (Nucleotide TA Repeat) PCR fragment analysis, revealing homozygous 7 TA repeats - (TA)7/(TA)7 in the promoter region of the UGT1A1 gene, suggesting a diagnosis of Gilbert syndrome.

With all these investigations, a final diagnosis of β-thalassemia intermedia with associated Gilbert syndrome was made. The patient was kept on regular follow-up, with monitoring of Hb and liver function, including serum bilirubin levels. The patient's laboratory parameters have been stable. Written informed consent was obtained from the patient and his family. Ethical clearance was obtained from the institutional ethical committee.

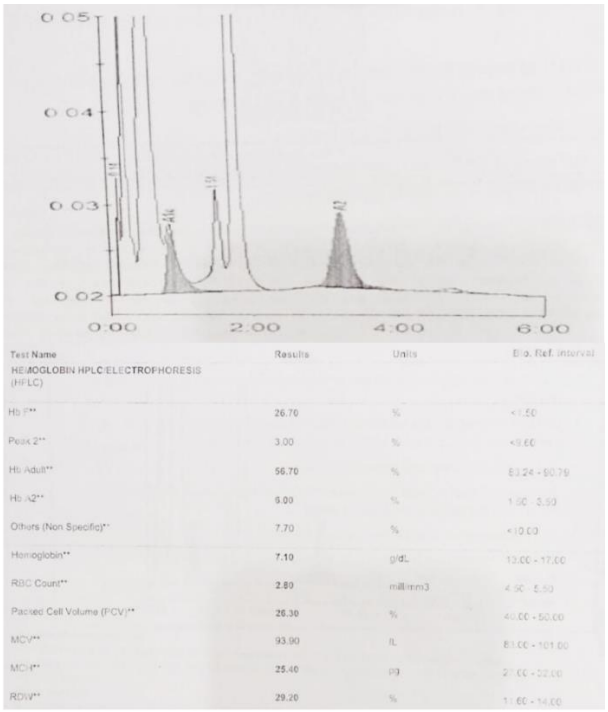

Figure 2: HPLC of the index case, illustrating elevated HbA2 (6%) and HbF (26.70%).

Table 1: Hematological and biochemical profile of the index case.

Parameters	15-07-2022	28-07-2022	13-08-2022	20-09-2022	09-11-2022
Total RBC count (millions/cumm)	**2.75**	3.56	3.74	4.25	4.9
Hb (g/dl)	**7**	**8.8**	**9**	**9.9**	**10.1**
PCV (%)	**21.7**	**28.3**	**29**	**32.4**	**30.9**
MCV (fl)	**78.9**	79.7	**77.8**	**74.6**	**63**
MCH (pg)	**25.6**	**24.72**	**24.06**	-	**20.5**
MCHC (g/l)	32.4	31.07	31	**23.29**	32.6
RDW (%)	**33.8**	-	-	-	**29.7**
TLC (thousand cell/cumm)	**3.87**	7.6	7.9	10.1	8.83
DLC (%)	N57 L37 E04 M02	N67 L26 E04 M03	N62 L32 E03 M03	N64 L30 E03 M03	N57 L34 E01 M08
Platelet count (thousand/cumm)	**90**	259	203	241	**107**
Reticulocyte count	**4.5**	-	-	-	-
SGOT (u/l)	24.6	-	37.4	47.8	36.5
SGPT (u/l)	14.9	-	21.7	31	16.8
Direct bilirubin	-	-	0.4	0.4	-
Indirect bilirubin	-	-	7.5	-	-
Total bilirubin (mg/dl)	4.3	-	7.9	7.8	**7.5**
Total protein (g/dl)	6.3	-	-	7.6	**6.1**

Discussion

Globally, it has been observed that around 7% of pregnant women carry beta or alpha zero thalassemia, HbS, C, D Punjab, or E, and over 1% of couples are at risk [1]. Patients with jaundice, growth retardation, and severe anemia presenting in early life and requiring lifelong blood transfusions are designated as thalassemia major (transfusion-dependent), resulting from a homozygous mutation of the beta-globin gene. Patients with homozygous β-thalassemia, who have milder anemia and require transfusions intermittently, are designated as thalassemia intermedia or minor (non-transfusion-dependent thalassemia) [2].

Thalassemia intermedia cases have increased serum bilirubin levels. Homozygosity for the extended (TA)7 sequence is known to be an important risk factor for hyperbilirubinemia and cholelithiasis in patients with thalassemia intermedia [4]. The (TA) repeat genotype influences serum bilirubin levels. The extended motif (TA)7 is associated with Gilbert syndrome. In a study by Tzetis et al., the (TA)7/(TA)7 genotype seen in Gilbert syndrome was found to be a modifying factor of bilirubin levels in thalassemic patients [6].

Gilbert syndrome, an autosomal recessive genetic disorder of bilirubin metabolism, results from a reduction of hepatic activity of bilirubin glucuronosyltransferase to about 30% of normal within the liver [7]. Reduced glucuronidation of bilirubin leads to unconjugated hyperbilirubinemia and recurrent episodes of jaundice. Gilbert syndrome is caused by certain common mutations, namely 211G → A (G71R), 524T → A (L175Q), 686C → A (P229Q), 1091C → T (P364L), and so on [8]. The reduced activity of the enzyme could result from a genetic defect in the UGT1A1 gene. Homozygosity for a defect in the TATAA box within the promoter region of the UGT1A1 gene leads to a mutation called UGT1A1*28 [9]. The molecular defect inserts an additional dinucleotide sequence (TA) into the transcription initiation sequence: A(TA)6TAA to A(TA)7TAA. It has been observed that homozygosity for the extended (TA)7 sequence is an important risk factor for hyperbilirubinemia in patients with beta-thalassemia intermedia [4]. The coinheritance of Gilbert syndrome is reported to elevate serum bilirubin levels and aggravate jaundice in patients with beta-thalassemia [4]. These fluctuating bilirubin levels can be attributed to various stress factors like fasting, dehydration, menstruation, and overexertion [5]. Although largely a benign medical condition that does not mandate any specific treatment, Gilbert syndrome in combination with thalassemia may result in severe hyperbilirubinemia and impact patient prognosis [6].

In concordance with the extensive literature search (Dabke PS [4], Tzetis M [6], Premawardhena A [7], and Canu G [8]), we would also like to emphasize the importance of properly evaluating the variable bilirubin levels observed in homozygous beta-thalassemia patients due to transfused red blood cell destruction rates, ineffective erythropoiesis, or hemolysis before arriving at a final diagnosis and deciding on the treatment plan for better patient prognosis.

Conclusion

In our case, increased serum bilirubin levels were observed due to the combined effect of hemolysis in beta-thalassemia and the association with Gilbert syndrome. The role of disproportionately increased bilirubin levels in our case was found to be related to UGT1A1 promoter polymorphisms. The results of the present study indicate that the (TA)7/(TA)7 genotype associated with Gilbert syndrome is a modifying factor of bilirubin levels when associated with beta-thalassemia. With this case report, we would

like to draw attention to the fact that gene modification plays an important role in the clinical variability of monogenic diseases.

Acknowledgments: None

Funding: None

Competing Interests: None

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