Challenges in diagnosis of Beta Thalassemia syndrome: The Importance of Molecular Diagnosis

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Abstract

Heterozygous Beta Thalassemia (β-thalassemia) patients generally are asymptomatic. However, if presented with intermediate phenotype, it is an uncommon and requires further investigations. We describe a 32-year-old woman Gravida 3 Para 2 with heterozygous β-thalassemia presented with symptomatic anaemia and had history of requiring frequent blood transfusion in each pregnancy. Physical examination was unremarkable. Laboratory results at presentation showed hypochromic microcytic anemia with reticulocytosis. Molecular study revealed intermedia phenotypes resulting from coinheritance of heterozygous β-globin chain mutation (IVS1-5) and a rare heterozygous α triplication ($\alpha\alpha\alpha^{anti\text{-}3.7}$). The laboratory diagnostic approaches and the challenges faced in investigating this case are discussed in the case report.

Keywords: alpha thalassemia, beta thalassemia, alpha triplication, beta globin chain, alpha globin chain

Introduction

Heterozygous β-thalassemia patients generally are asymptomatic and non-anemic compared to homozygous β-thalassemia patients who most of the time presented with severe form of anaemia and worse clinical presentations.[1]The molecular defects of β-thalassemia results in absence or reduce β-chain production while alpha chain (α-chain) synthesis is unaffected. As in homozygous β-thalassemia, excess α-chain leads to its precipitation in red cell precursors, forming an intracellular inclusion that leads to ineffective erythropoiesis and haemolysis. However, in heterozygous β-thalassemia, only minimal excess of α-globin chain produced resulting in mild anaemia without much of clinical significance.[2]

β-thalassemia exhibits remarkable phenotypic variability, ranging from mild condition to severe anaemia that requiring regular blood transfusion. The coinheritance of α-thalassemia results in milder clinical manifestations due to reduction in α-globin chain excess.[3] In contrast, additional of α-globin chain in β-thalassemia heterozygous increases the chain imbalance, converting a typically asymptomatic state of heterozygous β-thalassemia to thalassemia intermedia.[4]

We report a pregnant woman with thalassemia intermedia phenotype due to coinheritance of heterozygous β-globin chain mutation (IVS1-5) and a very rare heterozygous α globin triplication ($\alpha\alpha\alpha^{\text{anti-3.7}}$), which could not be identified by standard haemoglobin analysis. This case focuses on its clinico-hematological features and diagnostic approach which emphasizing the role of molecular testing in making the diagnosis.

Case Report:

A 32-year-old Malay woman, G3P2 at 25-week pregnancy presented with jaundice. She had history of multiple episodes of jaundice since secondary school and during her two previous pregnancies but was never being investigated further. She required 4-6 pints of red cells transfusion during each pregnancy.

Clinically, she was jaundiced and pale with no features of thalassemic face. There was no hepatosplenomegaly. The initial blood investigation results were; RBC count of 3.01×10^{12}/L, haemoglobin of 7.2 g/dL with MVC 74.2 fL, MCH 23.3pg and RDW 22.5%. Peripheral

blood smear showed hypochromic microcytic RBCs with anisopoikilocytosis, basophilic stippling, presence of normoblasts and polychromatic cells with reticulocytes count of 6%. Her serum ferritin was 190ug/L. Other investigations showed LDH of 287 U/L, total bilirubin of 111.7umol/L and normal liver enzymes. Coombs test was negative. Viral screening for Hepatitis B and C were negative.

The result of haemoglobin analysis by using SEBIA Hb Capillary Electrophoresis was consistent with β-thalassemia trait: HbA 91.2%, HbA_2 5.1% and HbF 3.7%. In view of her clinical presentation of chronic haemolysis and recurrent transfusions (thalassemia intermedia), further molecular studies; multiplex GAP PCR for α-gene deletion ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{MED}$, $-(\alpha)^{20.5}$ and $--^{THAI}$), multiplex Amplification Refractory Mutation System (ARMS) PCR for β gene mutation and multiplex PCR for α-globin gene mutation were done.[5] The molecular results of beta-globin gene revealed heterozygous IVS 1-5 (β^{+}) mutation. The result of DNA analysis for α-globin gene/cluster identified was a triple alpha globin chain ($\alpha\alpha\alpha^{anti-3.7}$) mutation (Figure 1). There was no deletional α thalasemia detected.

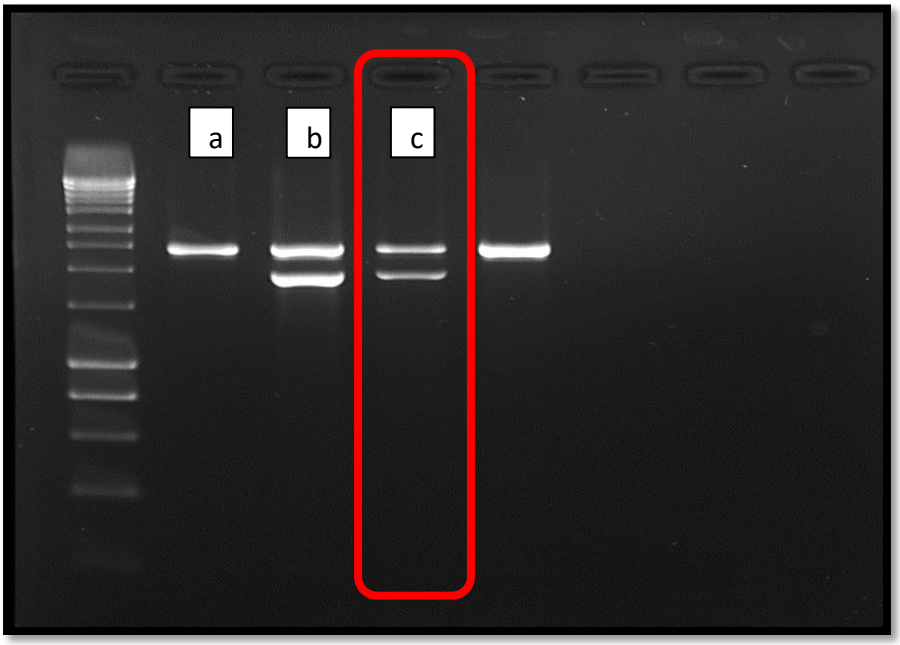

Figure 1: Alpha Triplication Multiplex PCR of negative control (a), positive control ααα-anti-3.7 (b) and the index patient (c). Presence of ααα-anti-3.7 in patient sample as in positive control

Discussion:

The genetic testing of the patient revealed the combination of triplication of α-globin gene with heterozygous β-thalassemia had produced a thalassemia intermedia phenotype, as it increases the imbalance between the α- and β-globin chains.

The deletional types of α-thalassemia are much more common worldwide compared to α-globin gene triplication.[6] The coexistence of α-gene triplication is an important modifier of the severity of β-thalassemia trait or β-thalassemia intermedia. It may exacerbate the phenotypic severity of β-thalassemia by causing severe anaemia with significant clinical manifestation by causing more globin chain imbalance.[7]

These genetic and clinical findings have important implications for prenatal screening and genetic counseling programs. The coinheritance of extra α-globin chain and β-thalassemia carriers are at risk of having an affected offspring, although the partners may be entirely normal. Thus, by detecting the α-globin gene triplication with β-thalassemia would definitely help physicians to provide appropriate genetic counselling and proper plan of management. This definitely will assist the physicians to illustrate the possible implications of the disease to the patients.[8]

This case is highlighted not only due to relatively rare α-gene triplication compared to α-gene deletion but also because of its co-inheritance with β-thalassemia trait that worsen the clinical and hematological features of the patient.

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