Hb E Syndrome with co-inheritance of Hb H-Constant Spring and Compound Beta Thalassaemia: A Diagnostic Challenge

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An 11- month-old girl with accidental findings of pale and hepatosplenomegaly. She was the last child of three siblings from a non-consanguineous marriage. The father and the mother were Hb E trait and Hb Constant Spring (Hb CS) trait respectively. Clinically the child was small for age with frontal bossing and hepatosplenomegaly. Sytemic examination was unremarkable. Her full blood picture showed moderate hypochromic microcytic anaemia with marked anisopoikilocytosis (Hb of 7.1g/dl, MCV of 44.6 fl, with MCH of 13.8 pg and RDW-CV of 24.0%). Quantitation of haemoglobin by using High Performance Liquid Chromatography (HPLC) and gel electrophoresis report showed that the patient has compound heterozygous E/ß$^{+}$ thalassaemia with Hb H-CS. She had increased of Hb A2/E (28.9%), and Hb F (11.2%) with presence of pre-run peak and a tiny peak at C window. Gel Electrophoresis by using agarose gel at alkaline pH discovered prominent A2 band and fast band to the left of Hb A band. H inclusions were positive. Further confirmation of diagnosis was done by molecular study. Alpha molecular study using Multiplex GAP PCR showed heterozygous --/SEA deletion (Fig. 1), while beta molecular study using Multiplex Amplification Refractory Mutation Systems (ARMS) revealed Cd 26 (G-A) and CAP +1 (A-C) mutations [Fig. 2].

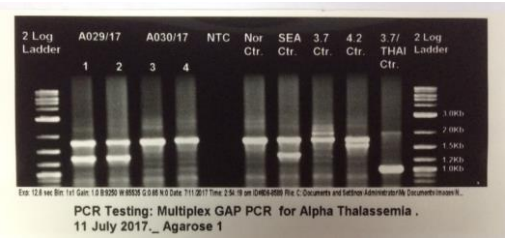

Fig. 1: Alpha molecular DNA analysis by multiplex GAP PCR of patient sample (lane 1 and 2) showed heterozygous --/SEA. Distinct band at 2503 bp, 1800 bp and 1349 bp.

Hemoglobin (Hb) E is common in Southeast Asia [1]. HbE disorders may be found heterozygous (AE) which usually asymptomatic, homozygous (EE) and compound heterozygous state with widely variable clinical features, ranging from transfusion

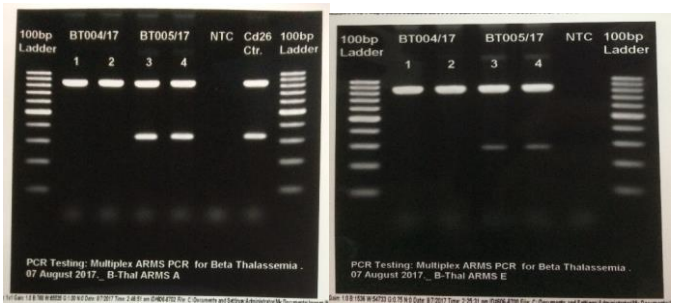

Fig. 2: Multiplex ARMS PCR for Beta Thalassaemia of patient sample (BT005/17) showed Cd26 (G-A) and CAP +1 (A-C) mutations.

dependence to a complete absence of symptoms [2]. Considering her history, clinical findings and investigations, the most likely diagnosis in our case is Compound heterozygous E/ß$^{+}$ thalassaemia with Hb H-CS. She had moderate hypochromic microcytic anaemia, raised Hb A_2/E and Hb F with presence of pre-run peak and a tiny peak at C window support the diagnosis. Unfortunately, we're unable to confirm the presence of Hb CS in view of no modalities available in our setting. However, with the family history of mother with Hb CS trait, the presence of Hb CS in this patient cannot be denied as a factor contributing to Hb H disease. Previous study reported Hemoglobin Constant Spring is often missed by routine laboratory testing, especially in subjects with co-inheritance of β-thalassaemia or β-variants. Hb CS detection clearly seen in capillary electrophoresis compared to HPLC [3]. As in this case only a very tiny peak of Hb CS noted on the HPLC. The molecular analysis for detection of Hb CS should be performed as for confirmation test. Hb H-CS has a severe phenotype than a deletional Hb H disease [4]. The diagnosis was confirmed by molecular analysis. Hence, genetic testing and family study are of particular importance to establish the exact genetic defect causing the abnormal Hb in this patient.

In view of thalassaemia is common in our region, it is important to identify complete genotyping to provide proper management, make clinical predictions and improve genetic counseling.

Keywords: Hb E syndrome, alpha, beta thalassaemia

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