

Compound Heterozygous Sickle Cell- β -Thalassemia: A Case Report from Upper Assam, India

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ABSTRACT

Sickle cell- β -thalassemia [Hb S/ β - thalassemia] is a rare type of hemoglobinopathy. The clinical characteristics of Hb S/ β - thalassemia are highly variable from a completely asymptomatic state to a severe disorder like homozygous sickle cell disease. In India IVS I-5 (G \rightarrow C) is the most common β -thalassemia allele. In this study we presented a case of compound heterozygous Hb S/ β - thalassemia

in a 14-year-old female with complaints of anemia with weakness, joint pain and splenomegaly. The patient and her parents were diagnosed by HPLC and for detection of mutational pattern of β -thalassemia; ARMS-PCR and DNA sequencing were performed. The HPLC report suggested that the patient have Hb S/ β - thalassemia and molecular diagnosis confirmed that the patient inherited IVS I-5 (G \rightarrow C) β - thalassemia mutation.

Keywords: Anemia, ARMS-PCR, DNA sequencing, HPLC

CASE REPORT

A 14-year-old female with complaints of anemia with weakness, joint pain and stomach pain presented in the Outpatient Department of Pediatrics. After taking informed consent systemic examination of the patient was done and the patient's spleen was found to be enlarged.

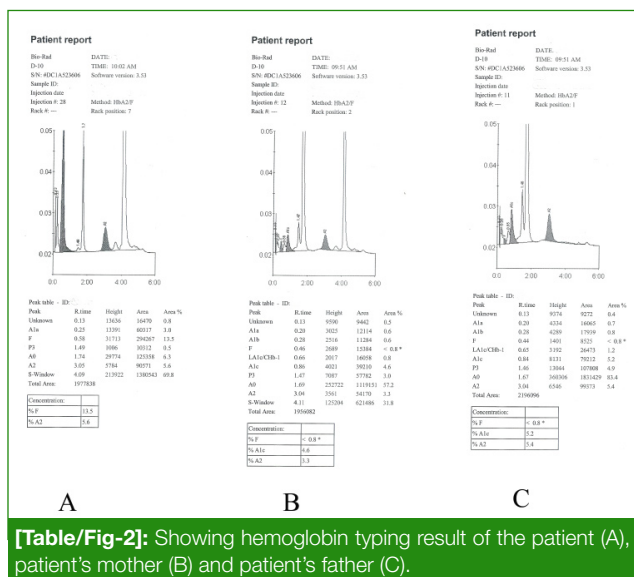
Blood samples of the patient and her parents were collected in EDTA vials after obtaining informed consent. Hematological data of the blood samples were determined on an automated blood cell counter (SYSMEX XS-800i, Japan) and the hemoglobin typing was performed by high performance liquid chromatography (HPLC) using the BIORAD D10 Hemoglobin Testing System. The hematological analysis showed that the patient was anemic [Table/Fig-1]. The hemoglobin typing results [Table/Fig-2] revealed that the patient was compound heterozygous for Hb S/ β - thalassemia, the father had β -thalassemia trait and the mother had sickle cell trait.

Molecular analysis was done to identify β - thalassemia mutation pattern associated with the patient. Genomic DNA was extracted by using QIAamp® DNA Blood Mini Kit (QIAGEN, Germany) for doing amplification refractory mutation system polymerase chain reaction (ARMS-PCR). ARMS-PCR was done following standard protocol [1]. Amplification was carried out in a 50 μ l reaction mixture containing 10x PCR buffer, 200 μ M dNTPs and 1.5mM MgCl₂. All the required primers, 2 μ l of DNA sample, 0.5U of

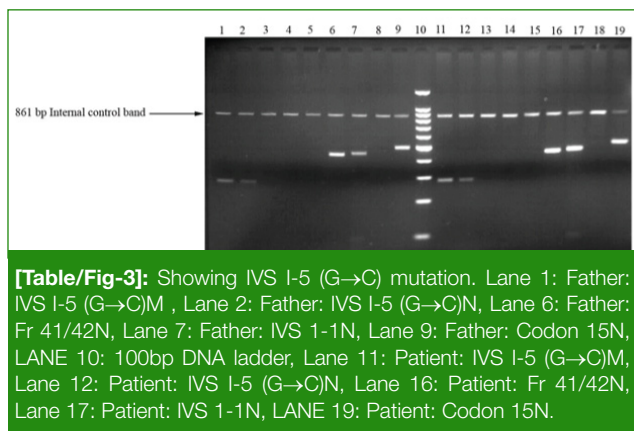
GoTaq® Flexi DNA Polymerase and nuclease free water were added to the reaction mixture. Amplification was carried out in an Arktik™ Thermal Cycler (Thermo Scientific, USA) as following sequence: 94°C for 5 minutes followed by 30 cycles of 94°C for 45 second, 65°C for 45 seconds and 72°C for 1.5 minutes, with a final extension at 72°C for 7 minutes. Then 15 μ l aliquot of the PCR product was separated on a 1.5% agarose gel in 1x Tris-borat-EDTA (TBE) buffer. The gel was stained with ethidium bromide and visualized under gel documentation system for the result. ARMS-PCR revealed that the β - thalassemia mutation pattern inherited by the patient was IVS I-5 (G \rightarrow C) mutation [Table/Fig-3]. The same mutation pattern was also found in the patient's father [Table/Fig-3]. Finally the PCR products were sequenced by automated DNA sequencer based on Sanger method to confirm the IVS I-5 (G \rightarrow C) mutation [Table/Fig-4].

Hematological Parameters	Patient	Father	Mother
Hb (g/dl)	5.9	12.0	12.2
MCV (fL)	75.00	69.90	88.50
MCH (pg)	23.00	22.60	29.10
MCHC (g/dl)	30.7	32.3	32.9
RBC $\times 10^6/\mu$ l	2.56	5.31	4.19

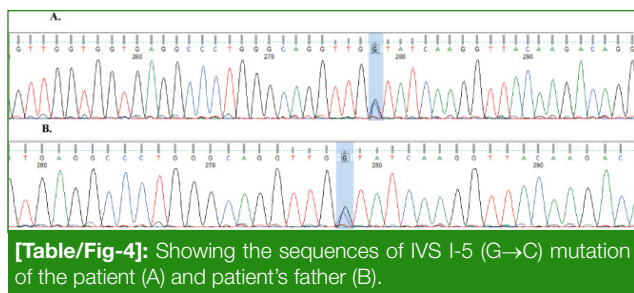
[Table/Fig-1]: Showing hematological parameters of patient and parents.



[Table/Fig-2]: Showing hemoglobin typing result of the patient (A), patient's mother (B) and patient's father (C).



[Table/Fig-3]: Showing IVS I-5 (G→C) mutation. Lane 1: Father: IVS I-5 (G→C)M, Lane 2: Father: IVS I-5 (G→C)N, Lane 6: Father: Fr 41/42N, Lane 7: Father: IVS 1-1N, Lane 9: Father: Codon 15N, LANE 10: 100bp DNA ladder, Lane 11: Patient: IVS I-5 (G→C)M, Lane 12: Patient: IVS I-5 (G→C)N, Lane 16: Patient: Fr 41/42N, Lane 17: Patient: IVS 1-1N, Lane 19: Patient: Codon 15N.



[Table/Fig-4]: Showing the sequences of IVS I-5 (G→C) mutation of the patient (A) and patient's father (B).

After complete diagnosis; folic acid course and 2 units of blood transfusion had been given to the patient. Follow-up of the patient had been done after confirmation of diagnosis along with genetic counseling.

DISCUSSION

Thalassemia and sickle cell disease have impacting on the population of low income countries [2]. The combination of the sickle cell mutation and β-thalassemia mutation results Hb S/β-thalassemia. In India 52 mutations accounted for 97.5% of all β-thalassemia alleles and IVS I-5 (G→C) is the

most common disease allele (54.7%) [3]. The prevalence of IVS I-5 (G→C) is 44.8% in the North and 71.4% in the East India [3].

Hb S/β-thalassemia disease is a hemoglobinopathy. Clinically, the disorder causes symptoms of moderate anemia and signs of sickle cell anemia. The incidence of β-thalassemia trait and sickle cell hemoglobinopathy in India varies between 3-17% and 1-44% respectively [4,5]. β-thalassemia is characterized by its genetic heterogeneity at the molecular level and more than 300 mutations of the β-globin gene have been characterized all over the world [6]. There are few β-thalassemia mutations which are common in India. Six mutations, codon 8-9 (+G), codon 15 (G→A), codon 41/42 (-TCTT), IVS 1-1 (G→T), IVS I-5 (G→C) and 619 bp deletion at 3' end of β-globin gene, account for about 80% of β-thalassemia mutation in Indian population [7].

Thalassemia and Sickle cell disease are two major erythrocytic genetic disorders prevalent in India. β-Thalassemia is predominantly found in the Mediterranean countries, the Middle East, Central Asia, India, North Coast of Africa and South America [8]. The prevalence of the sickle gene in India is found to vary from 2-34% [9]. The average frequency of Sickle cell disorders in India is 4.3% [10]. Sickle cell hemoglobin is mainly prevalent among the Tea garden communities of Assam [11]. The association of β-thalassemia with sickle cell hemoglobin is rare, which was reported as 1.7% [12].

For diagnosis of Hb S/β-thalassemia; hemoglobin typing by HPLC is very important. Elevated HbA2 is the most significant parameter in the identification of β-thalassemia carriers and the result should be interpreted together with other hematological and biochemical parameters [13]. HPLC is a method that allows rapid and precise detection of Hb variants, as well as sensitive quantitation of HbA2 [14]. When Hb S variant is present, percentage of Hb A2 and Hb F, family history, clinical data and hematological parameters help for distinguishing between homozygosis for Hb S and Hb S/β-thalassemia [15-18].

In the present study, ARMS-PCR and automated DNA sequencer has been used for detection of β-thalassemia mutations. ARMS-PCR has the advantage that it is theoretically possible to detect any known mutation [19].

When considering the findings associated with Hb S/β-thalassemia, blood transfusion might be the best treatment; however, many more Hb S/β-thalassemia case findings are necessary to more clearly identify an optimal treatment strategy.

CONCLUSION

Present study shows that the molecular techniques are very important for diagnosis of Hb S/ β -thalassemia. Correct diagnosis of Hb S/ β -thalassemia can be made based on high performance liquid chromatography (HPLC) testing, ARMS-PCR and DNA sequencing.

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