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Case Report

Genetics Section

Is Estimation of HbA2 Alone Sufficient for Screening Beta Thalassaemia Carriers: A Case in Perspective

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ABSTRACT

Beta thalassaemia is one of the most common inherited haemoglobinopathies, characterised by reduced or absent production of the beta globin chain. In India, the carrier frequency of thalassaemia is estimated to be 3-4%. The prevention of Beta thalassaemia is the best strategy, and this can be achieved through carrier screening and prenatal diagnosis. Carriers of beta thalassaemia can be easily identified using haematological parameters such as complete blood count and High Performance Liquid Chromatography (HPLC) for haemoglobin analysis. The characteristic findings observed in thalassaemia carriers include microcytosis, hypochromia, with a Mean Corpuscular Volume (MCV) of less than 80 fL and Mean Corpuscular Haemoglobin (MCH) of less than 28 pg. They also present with elevated levels of HBA2 ($\alpha 2\delta 2$) $\geq$3.5%. Carrier screening for beta thalassaemia primarily relies on the observation of elevated HbA2 levels. However, in rare cases, some carriers can have normal HbA2 levels, leading to false-negative screening results. In a case involving a married couple who underwent routine preconceptional screening by complete blood count and HPLC for thalassaemia screening, the male partner had elevated HbA2 levels (5.2%), while the female partner had normal HbA2 levels (1.6%). Molecular testing revealed that the male partner was heterozygous for the Intervening Sequence (IVS) 1-5 (G>C) mutation, while the female partner was found to be heterozygous for the CD41-42 (-CTTT) mutation. It is important to consider molecular testing of the HBB gene in couples, even if one partner is a carrier and the other partner has normal or borderline HbA2 levels.

Keywords: Anaemia, Heterozygous carrier, Molecular testing, Prenatal testing

CASE REPORT

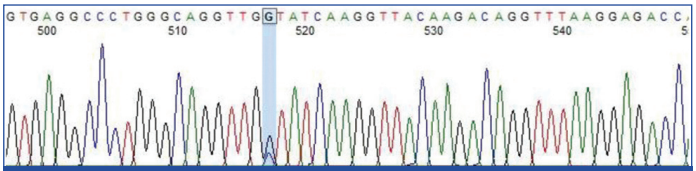
A non consanguineous couple from West Bengal came to the Department of Clinical Genetics for genetic counselling. They wanted to understand their risk of having a child with Beta thalassaemia. The couple had been trying to conceive for the past two years and were undergoing infertility evaluation. As part of the investigations, their complete blood counts and haemoglobin HPLC screening were conducted. The male partner's complete blood counts showed low MCV (62 fL) and low MCH (20.3 pg). HPLC revealed high HbA2 (5.2%), indicating that the male partner was a carrier of beta thalassaemia.

The female partner's complete blood counts also showed low MCV (66.9 fL) and MCH (20.8 pg), while HbA2 appeared to be within normal limits (1.6%) [Table/Fig-1]. To rule out iron deficiency anaemia, serum ferritin was checked, and it was found to be within the normal range (104.42 ng/mL). Based on the available reports, the male partner appeared to be a carrier, and it was not possible to completely rule out beta thalassaemia carrier status in the female partner. Further molecular testing was recommended to confirm her carrier status. The couple decided to undergo molecular testing for beta thalassaemia.

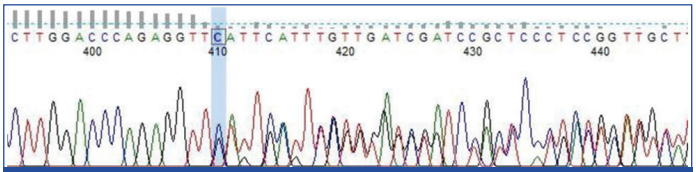
Parameters (range)	Male partner	Female partner
Haemoglobin (11.5-16.5) g/dL	12.2	10.1
RBC count (3.8-5.8) mil/cu.mm	6	4.86
MCV (80-96) fL	62	66.9
MCH (28-32) pg	20.3	20.8
MCHC (30-35) g/dL	32.8	31.1
RDW (11.5-14)%	16.9	16.4
HbA2 (2.0-3.5)%	5.2	1.6
Impression of HPLC	Beta thalassaemia trait	Normal

[Table/Fig-1]: Haematological parameters of the couple.

Genomic Deoxyribonucleic Acid (DNA) was isolated, and molecular testing was performed using Sanger sequencing. The male partner was found to be heterozygous for the common IVS 1-5 (G>C) mutation [Table/Fig-2]. Surprisingly, the female partner was also a carrier for the CD41/42 (-CTTT) mutation [Table/Fig-3]. This new information significantly changed the counselling approach. As both partners were carriers of beta thalassaemia, which is an autosomal recessive disorder, the couple was informed about the 25% risk of having an affected pregnancy and the importance of considering prenatal diagnosis for every pregnancy. Their extended family members were also counselled to undergo screening for beta thalassemia by Sanger sequencing of the known familial variant.



[Table/Fig-2]: Sanger sequencing of the male partner showing IVS 1-5 (G>C) heterozygous mutation.



[Table/Fig-3]: Sanger sequencing of the female partner showing CD41/42 (-CTTT) heterozygous mutation.

DISCUSSION

Haemoglobinopathies are common monogenic disorders, and India has the largest number of children with thalassaemia in the world [1]. Prevention of the disease is much more cost-effective

than lifelong treatment through blood transfusions, iron chelation, or Bone marrow transplantation for the affected children. Prevention beta thalassaemia can be achieved by raising awareness about the disorder, screening for heterozygous carriers, discouraging marriages between carriers, and conducting prenatal testing for pregnancies likely to be affected [1].

Beta thalassaemia is unique among genetic disorders because heterozygous carriers can be easily identified using haematological parameters instead of genetic testing [2]. Carriers do not show clinical symptoms, but their red blood cell indices exhibit microcytosis (MCV <80 fL), hypochromia (MCH <28 pg), and HbA2 levels ≥3.5%. Among these, slightly elevated HbA2 levels ≥3.5% serve as a key screening marker for beta thalassaemia carriers and must be accurately measured to successfully identify carriers [2,3]. However, in some beta thalassaemia carriers, HbA2 levels can remain within the normal range, as seen in the current case (HbA2-1.6%), potentially leading to missed detection in screening programs.

Normal HbA2 levels typically range between 2.2% and 3.5% [4]. Beta thalassaemia carriers experience increased HbA2 levels due to reduced beta chain synthesis. However, the synthesis of other chains remains unaffected, and more delta chains are available to combine with alpha chains [5]. Elevated HbA2 levels in normal individuals can occur in conditions that delay the maturation of young red blood cell precursors, such as megaloblastic anaemia, certain anti-Human Immunodeficiency Virus (HIV) medications like nucleoside reverse transcriptase inhibitors, megaloblastic anaemia caused by folate or Vitamin B12 deficiency. Rarely, carriers may have HbA2 levels within the normal range when there is concomitant iron deficiency anaemia, mild beta thalassaemia β^{+} or β^{++} variants, α thalassaemia, δ thalassaemia, or $\delta\beta$ thalassaemia resulting from deletions in HBB and HBD genes. Some beta thalassaemia mutations associated with borderline HbA2 values and mild phenotypes include CAP+1 (A>C)-101 (C>T), -92 (C>T), PolyA (-AT), PolyA (A>G), PolyA (-AA), PolyA (T>C), IVS-I-6 (T>C), IVS-II-844 (C>G), +1480 (C>G), and +33 (C>G) [6]. Recently, mutations in KLF1 and BCL11A were also identified as causative factors for borderline HbA2 levels [7].

In the current case, the male partner displayed typical haematological features of a beta thalassaemia carrier, such as microcytosis, hypochromia, and elevated HbA2. The identified mutation was IVS1-5 (G>C), which causes a severe β^{0} phenotype and is the most common mutation in India. The G>C substitution at the fifth base of IVS1-5 activates three cryptic donor sites and disrupts normal splicing. It is commonly observed in Asian Indian, Pakistani, and Southeast Asian populations. HbA2 levels associated with this mutation range between 4.2% and 6.1% [8].

The female partner also exhibited hypochromia and microcytosis, but her HbA2 level was within the normal range (1.6%). Iron deficiency anaemia can lower HbA2 levels as it reduces the synthesis of alpha globin chains more than non alpha chains. Beta chains compete more efficiently with available alpha chains than delta chains, leading to a decrease in HbA2 levels [9]. However, serum ferritin levels in the female partner were normal, ruling out iron deficiency as the cause. As the male partner had beta thalassaemia trait, molecular testing was performed on the female partner, revealing that she was a carrier for the CD41/42 (-CTTT) mutation. This mutation also causes a β^{0} phenotype and is a deletion point mutation that affects messenger Ribonucleic acid (mRNA) translation. Mean HbA2 levels associated with this mutation range between 3.7% and 6.8% [8]. Colaco S et al., reported eleven beta thalassaemia mutations in individuals with borderline HbA2 values between 3% and 3.9%, with one individual carrying the CD41/42 (-CTTT) mutation and an HbA2 level of 3.3% [10]. Nadkarni AH et al., identified 131 beta thalassaemia heterozygotes with HbA2 levels between 1.0% and 3.9% who were heterozygous for eight different beta thalassaemia mutations, but CD41/42 (-CTTT) was not among the reported mutations [11]. A study by Gorivale M et al., revealed that among 2.5% of 967 couples who opted for prenatal diagnosis, one partner had normal or borderline HbA2 values that could have been missed by conventional screening. The mutations observed in their cohort were CAP +1 (A>C), IVS1-5 (G>C), Poly A (T>C), CD30 (G>C), CD15 (G>A), and CD16 (-C) [12]. However, the CD41/42 (-CTTT) mutation observed in the female partner in this case with normal HbA2 levels seems to be the first report of a beta thalassaemia carrier with this mutation. However, factors like δ gene deletion, α gene mutation, and mutations in modifier genes like KLF1 could not be assessed in present study.

Therefore, it is crucial to remember that individuals with normal HbA2 levels can still be a carriers for beta thalassaemia. In couples where one partner is a beta thalassaemia carrier, both partners should undergo molecular testing of the HBB gene.

CONCLUSION(S)

Carrier screening and prenatal diagnosis are the most effective methods for prevention of beta thalassemia, and elevated HbA2 levels, along with red blood cell indices, usually help to identify thalassaemia carriers. However, in some cases, HbA2 levels can be normal, leading to false-negative results in screening and the birth of a child with thalassaemia major. Therefore, physicians should maintain a high level of suspicion and consider molecular testing in couples even if only one partner is a beta thalassaemia carrier. Molecular testing should also be considered in situations where partners have borderline HbA2 levels.

REFERENCES

[1] National Health Mission Guidelines on Haemoglobinopathies in India. Prevention and Control of Hemoglobinopathies in India. [book auth.] Ministry of Health & Family Welfare Government of India: National Health Mission. 2016.
[2] Giambona A, Passarello C, Renda D, Maggio A. The significance of the hemoglobin A(2) value in screening for hemoglobinopathies. Clin Biochem. 2009;42(18):1786-96. Doi: 10.1016/j.clinbiochem.2009.06.026.
[3] Origa R. β-Thalassaemia. Genet Med. 2017;19(6):609-19. Doi: 10.1038/gim.2016.173.
[4] Sharifi A, Mahdieh N. HBB mutations and HbA2 level: Escaping the carrier screening programs. Clin Case Rep. 2021;9(2):973-77. https://doi.org/10.1002/ccr3.3714.
[5] Needs T, Gonzalez-Mosquera LF, Lynch DT. Beta Thalassaemia. [Updated 2023 May 1]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK531481/.
[6] Stephens AD, Angastiniotis M, Baysal E. ICSH recommendations for the measurement of haemoglobin A2. Int J Lab Hematol. 2012;34(1):01-13. Doi: 10.1111/j.1751-553X.2011.01368.x.
[7] Hataichanok S, Thawinan W, Fucharoen G. Thalassaemia and erythroid transcription factor KLF1 mutations associated with borderline hemoglobin A2 in the Thai population. Archives of Medical Science. 2020;18(1):112-20. Doi: 10.5114/aoms.2020.93392.
[8] Kountouris P, Lederer CW, Fanis P, Feleki X, Old J, Kleanthous M. IthaGenes: An interactive database for haemoglobin variations and epidemiology. PLoS ONE. 2014;9(7):e103020. Doi: 10.1371/journal.pone.0103020.
[9] Passarello C, Giambona A, Cannata M. Iron deficiency does not compromise the diagnosis of high HbA(2) β thalassaemia trait. Haematologica. 2012;97(3):472-73. Doi: 10.3324/haematol.2011.052357.
[10] Colaco S, Colah R, Nadkarni A. Significance of borderline HbA_2 levels in β thalassaemia carrier screening. Sci Rep. 2022;12:5414. https://doi.org/10.1038/s41598-022-09250-5.

[11] Nadkarni AH, Gorakshakar AC, Sawant PM. The phenotypic and molecular diversity of hemoglobinopathies in India: A review of 15 years at a referral center. Int J Lab Hematol. 2019;41(2):218-26. Doi: 10.1111/ijlh.12948.

[12] Gorivale M, Sawant P, Mehta P, Nadkarni A. Challenges in prenatal diagnosis of beta thalassaemia: Couples with normal HbA2 in one partner. Prenat Diagn. 2015;35(13):1353-57. https://doi.org/10.1002/pd.4706.

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