Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Pediatric Hematology Oncology Journal 7 (2022) 74–76

Contents lists available at ScienceDirect

Pediatric Hematology Oncology Journal

journal homepage: https://www.elsevier.com/journals/pediatric-hematology-oncology-journal/

Dutch-beta thalassemia: A rare mutation from India

Nirali Sanghvi*, [1], Priyanka Aggarwal, Vineeta Singh, Vineeta Gupta

Division of Pediatric Hematology-Oncology/BMT, Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, 221005, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 27 January 2022
Received in revised form
20 May 2022
Accepted 7 June 2022
Available online 9 June 2022

Keywords:
COVID-19
Transfusion dependent thalassemia
Iron chelation therapy
Deferasirox

ABSTRACT

Introduction: COVID-19 pandemic imposed challenges towards management of transfusion-dependent thalassemia patients (TDT). The need for regular blood transfusions and iron chelation therapy in these patients added further uncertainty about managing COVID-19 in this subset of patients.
Aims: To describe the clinical manifestations of SARS-CoV2 infection in patients with TDT and to evaluate feasibility of home management for patients with mild disease.
Materials and methods: The study involved TDT patients registered with thalassemia day care center, DMCH, who tested positive for COVID-19 by RTPCR. The demographics, clinical characteristics and baseline investigations were recorded. Patients with mild disease were managed at home and others were hospitalized. The daily home monitoring and the hospital course were noted and analyzed.
Results: The study involved 14 TDT patients who were infected with SARS-CoV2 with a mean age of 18.9 ± 6.7 years and a male to female ratio of 6:1. Five patients each were in low and high-risk groups and 4 patients were in highest risk group. The symptoms reported by these patients were fever, fatigue, sore throat etc. Two patients were hospitalized with one patient requiring oxygen therapy. He was discharged after 48 hours. The other patient had severe cardiac iron overload and diabetes mellitus. His iron chelation therapy was with held during hospitalization. He presented with a cardiac arrhythmia later and was cardioverted. Thus, all other patients were continued on iron chelation with deferasirox. Twelve patients were successfully managed at home with regular telephonic monitoring.
Conclusion: Patients with thalassemia do not necessarily need hospitalization for management of COVID-19. Home management can be offered to patients with mild disease in a resource limited setting. Iron chelation with deferasirox can be continued safely.

1. Introduction

Beta Thalassemia is a hereditary blood disease caused by numerous mutations in the *HBB* gene [1]. Mutations in this gene can result in variable β-globin production leading to autosomal recessive disorders like β-Thalassemia (quantitative β-chain defect) and sickle cell anemia (qualitative β-chain defect). Although multiple mutations are known to occur with β-Thalassemia, the common ones in Indian population are only few which constitute about 90% of them. Nonetheless, many rare variants of the disorder are also known like the Dutch 12.6 kb β^0-thalassaemia deletion. We hereby present a case report of a child with Thalassemia with rare mutation with the consent of the parents.

2. Case report

An eight year old female child presented to us with a history of progressive paleness of body and jaundice for 15 days. She had no previous history of blood transfusion. On physical examination, she had moderate pallor, icterus and palpable spleen 3 cm below the

* Corresponding author. Division of Pediatric Hematology-Oncology, Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Lanka, Varanasi, 221005, India.
E-mail addresses: drniralis@yahoo.co.in (N. Sanghvi), tamanna.horizon@gmail.com (P. Aggarwal), vinesingh93@gmail.com (V. Singh), vineetaguptabhu@gmail.com (V. Gupta).
Peer review under responsibility of Pediatric Hematology Oncology Chapter of Indian Academy of Pediatrics.
[1] Permanent Address: Jain Pathology Laboratory and Blood Bank, Gandhi Chouraha, Mandsaur, MP, India. Pin code: 458001.

https://doi.org/10.1016/j.phoj.2022.06.001

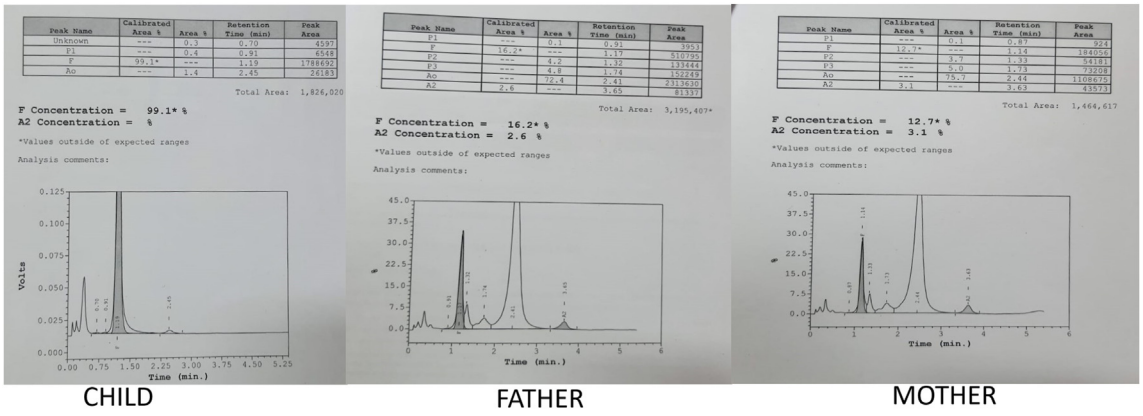

Fig. 1. Hplc of the family.

Table 1
Comparative data on the laboratory parameters of the index case and parents.

PARAMETER	CHILD	MOTHER	FATHER
Hb (gm/dL)	9.3	10.5	14.7
MCV (fL)	63.3	67.9	69.3
MCH (pg)	18.5	21	22.8
MCHC (%)	29.5	31	33
RDW (%)	21.7	18.0	15.9
RBC Count (10^6/μL)	5.02	4.99	6.44
HbF (%)	99.1	12.7	16.2
HbA (%)	–	75.7	72.4
HbA_2 (%)	–	3.1	2.6

left costal margin along its long axis. Rest of the examination was unremarkable. She had a haemoglobin level of 9.3 gm% with normal total leucocyte count and platelet count. The RBC indices in the order of MCV, MCH, MCHC, RBC count and RDW were as follows: 63.3 fL, 18.5 pg, 29.5 g/dL, 5.02 million cells/cmm and 21.7% respectively, drawing an interpretation of microcytic hypochromic blood picture. Her iron profile was normal. Therefore a high performance liquid chromatography (HPLC) was performed on BIO-RAD Variant II analyser, which revealed 99% HbF with absence of HbA and A_2 (Fig. 1). Subsequently, an HPLC of the parents was also performed which revealed that both had elevated levels of HbF (12.7% and 16.2% in mother and father respectively) with normal HbA_2 levels. A comparative table of their laboratory parameters are depicted in Table 1.

Thus in view of the clinical and laboratory parameters of hemolytic anemia with elevated HbF levels and complete absence HbA possibilities of: 1) homozygous delta-beta (δβ) Thalassemia, 2) homozygous hereditary persistence of fetal haemoglobin, 3) double heterozygous δβ Thalassemia and classical β Thalassemia; and 4) rare variants of homozygous β Thalassemia were considered. Hence, a comprehensive beta Thalassemia [*HBB*] gene analysis by next generation sequencing (NGS) was done. This included selective amplification and sequencing of the targeted region of the genome/genes along with multiplex PCR amplification to create a target amplicon library from each DNA sample. A deletion duplication analysis was also performed. This assay enabled target specific library generation for NGS analysis of HBB gene. It revealed a homozygous deletion spanning *HBB* gene from upstream of exon 1 to exon 3 downstream within the detection limits copy number variants (CNV) in the *HBB* gene of this subject. This deletion is known as the Dutch I β°- Thalassemia and it is a homozygous β°-Thalassemia.

Currently the patient is non-transfusion dependent and receiving folic acid supplementation.

Due to financial constraints, gene analysis of the parents could not be performed to confirm their heterozygous state.

3. Discussion

Beta Thalassemia is an inherited blood disorder caused by over 350 mutations in the *HBB* gene which is responsible for the synthesis of a protein called beta-globin, a subunit of haemoglobin [1]. Mutations in this gene can result in decreased (β^+) or no (β^0) β-globin production leading to autosomal recessive disorders like β-Thalassemia and sickle cell anemia. In a meta-analysis from India, information on 8505 alleles has been collated and 64 β-globin gene mutations causing β –Thalassemia have been identified [2]. Nationally, IVS1-5 G > C is the single most common mutant allele and represents 54.7% of all β-thalassemia mutations reported. Five common mutations found in Indian sub-continent, which comprise 82.5% of all mutations, are: 1) IVS1-5 G > C, 2) IVS1-1 G > T, 3) codon 41/42(-TTCT), 4) codon 8/9 (+G) and 5) the 619 base-pair (bp) deletion [2,3]. Other 5 mutations namely: Codon 15 G > A, Codon 30 G > C, Cap site +1 A > C, Codon 5(-CT) and Codon 16(-C) account for an additional 11.0% of all mutant alleles [2] These constitute about 90% of the mutations found in India, however many rare variants of the disorder may also be reported sporadically.

The Dutch 12.6 kb β^0-thalassaemia deletion is a member of a discrete 12–13 kb size category of deletions [4,5]. It is a homozygous deletion spanning *HBB* gene from upstream of exon 1 to exon 3 downstream detected in the *HBB* gene [12612 NTS deleted in the beta gene]. There is no beta chain production and considerable increase in gamma chain formation [5]. Individuals with Dutch I β °- Thalassemia show few clinical symptoms like mild to severe anemia in homozygous condition and elevated fetal haemoglobin (4–11%) in heterozygous condition [4,5]. The presence of high HbF levels may also postpone its diagnosis beyond infancy. Also, the higher levels of HbF lead to an increased oxygen carrying capacity of the RBCs. Therefore despite the absence of HbA the patient has only mild anemia and is not transfusion dependent.

A striking finding of normal HbA2 with an elevated HbF in the parents has been seen in this patient, although in most beta thalassemia heterozygotes with deletion of the beta globin gene, the HbA2 is unusually elevated. Expression of the adult β globin gene depends on lack of competition from the upstream γ gene for LCR

sequences. It has been proposed that deletion of the β promoter removes competition for the upstream βLCR and limiting transcription factors. This permits greater interaction of the LCR with the *cis* δ and γ genes, thus resulting in their enhanced expression [6]. In homozygote patients of Dutch β-thalassaemia there is a high level of $^{G}\gamma$ chain in Hb F whereas in heterozygotes the Hb F value ranges 4–11% [4,5]. This would explain the cause of high Hb F level in the parents, however a suitable explanation of normal Hb A2 in them was not found.

The Dutch beta variant has ***not*** yet been reported from Uttar Pradesh or India all together and there are only anecdotal case reports since 1987 from other nations as well [3,7]. This deletion has been previously reported in isolated cases of Dutch β °- Thalassemia in different ethnic population specifically in autochthonous Dutch population [8].

HBB genetic testing must be employed for diagnostic purposes in individuals with clinical symptoms of β-Thalassemia or a hemoglobinopathy. Parents who have symptoms, family history of the disorder, or are known carriers of the disease, can benefit from prenatal testing for mutations in this gene.

4. Conclusion

A high level of HbF on HPLC is more commonly associated with homozygous delta-beta Thalassemia or homozygous hereditary persistence of fetal haemoglobin but one should also consider a possibility of homozygous beta Thalassemia with rare genetic mutations which can be confirmed by mutational analysis. Thus we reiterate the importance of mutational analysis in cases of markedly elevated HbF levels beyond fetal period.

Financial support

None.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Patient's consent:

Consent has been obtained from the patient's parents.

Declaration of competing interest

The authors have no conflict of interest to declare.

References

[1] Jaing TH, Chang TY, Chen SH, Lin CW, Wen YC, Chiu CC. Molecular genetics of β-Thalassemia: a narrative review. Medicine (Baltim) 2021;100(45):e27522.
[2] Sinha S, Black ML, Agarwal S, et al. Profiling β-thalassemia mutations in India at state and regional levels: implications for genetic education, screening and counselling programmes. HUGO J 2009;3(1–4):51–62.
[3] Panigrahi I, Marwaha RK. Mutational spectrum of thalassemias in India. Indian J Hum Genet 2007;13:3636–7.
[4] Gilman J. The 12.6 kilobase DNA deletion in Dutch β°-thalassaemia. Br J Haematol 1987;67(3):369–72.
[5] Gilman JG, Abraham J. DNA sequence analysis of the Dutch beta zero-Thalassemia deletion. Biomed Biochim Acta 1987;46(2–3):S131–5.
[6] Thein S. The molecular basis of β -thalassemia. Cold Spring Harb Perspect Med 2013;3:a011700.
[7] Globin.bx.psu.edu. HbVar ID 987 [online] Available at: https://globin.bx.psu.edu/hbvar/hbvar.html. [Accessed 5 January 2022].
[8] Giordano PC, Harteveld CL, Heister AJ, Batelaan D, van Delft P, Plug R, Losekoot M, Bernini LF. The molecular spectrum of beta-Thalassemia and abnormal hemoglobins in the allochthonous and autochthonous Dutch population. Community Genet 1998;1(4):243–51.