

**Case Report**

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Haemoglobin D Iran With Beta Thalassemia in A Primigravida With Anaemia

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Anaemia is quite common in female population of developing countries including Pakistan. Beside haemoglobinopathies are often seen in India, Pakistan and Iran due to traditional practices of consanguineous marriages. Haemoglobin D-Punjab is one of the most common subvariants (55%) of Haemoglobin D, which can be inherited as a homozygous or a heterozygous trait with other haemoglobinopathies. Although, Haemoglobin D-Punjab is commonly seen but another variant of HB D like Hb D Iran with heterozygous trait of β thalassemia is rarely reported. We present a rare case of co-inheritance of Haemoglobin D- Iran and β thalassemia in a young primigravida of Kashmiri origin in her twenties. She was six months pregnant and was diagnosed to have anaemia. She had history of diarrhea. On examination, she was pale looking and mildly icteric. Systemic examination was insignificant except for mild splenomegaly. The fundal height was normal for age to date. On investigation, she had mild anaemia with microcytic hypochromic blood picture. Hemoglobin electrophoresis showed a band of Hb A₂+Hb F+S/D. Molecular studies of combined Haemoglobin D –Iran and beta thalassemia. She was counseled about his disease and advised follow-up up at 6 months after delivery after delivery.

Keywords: Anaemia; Haemoglobinopathy; Primigravida; Thalassemia

Introduction

Hemoglobin D (Hb D), is a variant of hemoglobin that occurs mainly in north-west India, Pakistan and Iran [1]. The replacement of glutamic acid with glutamine in normal Hemoglobin A at 121 position on its beta chain leads to a new structurally different variant of haemoglobin called Hb D [2]. Hb D is clinically silent in its heterozygous state, but coinheritance of Hb D with either Hb S or thalassemia produces clinically significant conditions like sickle cell anemia and chronic hemolytic anemia of moderate severity [3].

The Hb D Iran trait and homozygous cases have been already reported in the literature [4,5]. However, few studies have reported compound heterozygotes of Hb D Iran with other Hb variants [6]. We report a rare case of compound heterozygous condition of Hb-D β thalassemia trait in a Kashmiri family living in Azad Kashmir, Pakistan.

Case Report

A young six-month prime gravida of Kashmiri origin from Muzaffarabad, Azad Kashmir Pakistan in her twenties presented with chronic diarrhea with history of chronic anemia. She was nonsmoker, nonalcoholic. There was no past history of parasitic infestation, blood loss, weight loss, tuberculosis, any other disease.

She was treated by various GPs with oral hematinics without much improvement of her symptoms & correction of anaemia. She was believed to have diarrhea due to iron therapy and was lately administered parenteral iron therapy. However, her Hb remained 9gm/dl and did not improve. On examination, she was conscious and oriented in time place and person. Her BP was 100/70mm Hg and pulse rate of 80 beats per minute. She was fair colored with mild jaundice. There was no cyanosis, clubbing, koilonychia, lymphadenopathy or edema. Systemic examination was normal except for mild splenomegaly. Ultrasound abdomen showed 24 weeks fetus, normal sized liver and an enlarged spleen of 5cms in size. Her stool examination revealed cysts of *Entamoeba histolytica*. She was treated with tablet metronidazole 800mg tid for 10 days. Her diarrheal symptoms disappeared and follow up stool examination was normal. Retrospectively, her family history revealed consanguine marriage of her parents. Complete blood picture showed Hb of 8.8g/dl, WBC count of 13.5×10^3 /ul with 68% polymorphs. RBC count was 5.16×10^6 /ul. MCV was 60.8 fL, MCH 17.1pg and HCT 31.4%. Platelets were 232×10^3 /ul. Peripheral blood film showed microcytic hypochromic blood picture with moderate anisopoikilocytosis and a few target cells. Reticulocyte

count was 5.5%. Hemoglobin electrophoresis was advised to rule out haemoglobinopathy. It showed a band of Hb A2 plus Hb F plus Hb S/ D. Hb F was 0.8% and H A2 3.1%. Sickling test was negative. PCR for thalassemia showed a mutation in Fr 8-9(+G) and another Hb D (Iran). A diagnosis of combined haemoglobin D -Iran and β thalassemia were made. Urine examination was normal. The diagnosis was conveyed to the patient. No intervention was needed, however, she was counselled regarding the condition and advised to follow up at 6 months after delivery for blood screening for haemoglobinopathy.

Discussion

Hemoglobinopathies are one of the common blood disorders seen in Pakistani population. Mutations in the globin genes can cause either a quantitative reduction in output from that gene or alter the amino acid sequence of the protein produced. Quantitative defects cause thalassemia, whereas qualitative changes are referred to as Hb variants. These hereditary disorders of Hb pose a massive health problem in many third world countries including India, Pakistan, and Iran [7].

In a retrospective study conducted at National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan, a total of 2739 patients blood samples were analyzed from 2010-2014. The overall frequency of hemoglobinopathies was 34.2%, among which beta Thalassemia minor was 51.8% and Hb D trait was 6.7% and Beta Thalassemia major was 24.1%.7 Nearly similar findings were observed in other studies conducted by in Pakistan [8,9].

Thalassemias are inheritable common genetic disorder worldwide due to alteration in haemoglobin (Hb) production. About 4.83% of world's populations carries of globin chain variants including 1.67% of the population which is heterozygous for α -thalassemia and β -thalassemia [7].

Beta thalassemia occurs as a result of mutation in Hb- β gene on chromosome-11 and follows autosomal recessive pattern of inheritance. It spreads from asymptomatic to symptomatic depending on genetic conditions [4]. β thalassemia represents a heterogeneous group of hemoglobin disorders and is caused by

reduced or absent beta globin gene expression. The disease has high frequency in Mediterranean regions, Africa, Southeast Asia and Indian subcontinent. It is estimated that there are 270 million carriers of thalassemia world over and 80 million of them have beta thalassemia traits. It is a significant health problem in Pakistan and 5-7% of our population (approx 9-13 million) has thalassemia minor [10].

Hb-D is a haemoglobin variant is seen mainly in North West India, Pakistan and Iran [4]. The co-inheritance of Hb-D and β thalassemia minor is rarely reported [11].

Hb D was first reported as a new Hb by Itano in 1951 in a North American family [12].

Hb D Iran reported by Rahbar in a family in central part of Iran is the replacement of glutamic acid by glutamine at 22 (helical residue B4) [13]. In a study carried out at a largest medical center in Iran from November 2002 to December 2010, hemoglobin analysis of 220 patients with Hb D variants showed 92 cases of Hb D Punjab and 88 patients with Hb D Iran. There are few studies in literature regarding prevalence of Hb D in different countries [13]. There is scarcity of data regarding compound heterozygous Hb D Iran with β Thalassemia trait. Hb D occurs in four forms: heterozygous Hb D trait, Hb D- thalassemia, Hb S-D disease and homozygous Hb D disease, amongst them Hb D-Punjab is by far the commonest [3].

During 22 years of thalassemia screening in a Thalassemia Prevention Unit in northern Greece that covers the regions of central and western Macedonia, northern Greece, with a population of around 2.5 million, only 30 cases were found heterozygous of Hb D, 1 case of compound heterozygous for Hb S / Hb D-Punjab and 1 case of compound heterozygous for β -thalassemia / Hb D-Punjab among 80,401 subjects screened.

β thalassemia trait is very common and is found in more than half of pregnant anaemic patients in our region. Various studies have reported that the quantity of Hb D Iran eluting in the Hb A2 window in high performance liquid chromatography varies from 36.0 to 47.7% in a heterozygous condition, while in compound heterozygous states, the quantity varies between 47.3 and 94.4%.

Table 1: Hematological profile.

Blood Counts			
TLC	12.21		4-109/L
RBC	4.67		3.8-5.8X 10 ¹² /L
Hb	8.8		12-15g/dl
PCV	0.28		0.37-0.47l/L
MCV	56.7		76-96fL
MCH	18.8		27-32pg
MCHC	32.1		31.5-34.5g/dl
Platelet count	178		150-400X 10 ⁹ /L
Reticulocyte count	4.7		0.5-2.5%
Red cell width distribution	37.3		39-46fL
Differential leucocyte count			
Neutrophils	66		40-60%

Lymphocytes	22	20-40%
Monocytes	8	02-10%
Eosinophils	4	1-6%
Red Cell Morphology		
Hypochromia	++	4-109/L
Microcytosis	++	3.8-5.8X 1012/L
Anisocytosis	+	12-15g/dl
Poikilocytosis	+	0.37-0.47l/L
Target cells	+	76-96fL
Tear drop cells	Occasional	27-32pg
Haemoglobin studies		
Haemoglobin electrophoresis shows	Band of Hb A2+Hb F+S/D	
Haemoglobin F quantitation	0.80%	
Haemoglobin A2 quantitation	3.1	2.0-3.5%
Sickling/ Sickle cell solubility	Negative	
PCR	Shows Hb-D (Iran)/ Beta thalassaemia (Fr 8-9)	
Opinion	Hb-D (Iran)/ Beta thalassaemia Hb-D 96.1%	

Hb D-Iran is commonly found to be co-existent with β -Thalassaemia and generally doesn't get diagnosed until patient comes for the diagnosis or treatment of the Thalassaemia. In homozygous cases, such as one reported by Thornburg CD et al., Hb D-Iran can present with anaemia, poikilocytosis and mild hemolysis [5]. When co-existing with β -Thalassaemia, the patient's clinical symptoms arise due to the thalassaemia component and not due to the Hb D-Iran counterpart. One such case has been reported by Agrawal MG et al., where Hb D-Iran was co-existing with β -Thalassaemia [14]. In heterozygous form with Hb A (Hb D-Iran trait), the patient is usually clinically silent and goes undetected as is evident from this index case (Table 1). Another case was reported by Gupta et al., where Hb D-Iran was present along with Hb D-Punjab [15]. It should be noted that in all these cases, Hb D-Iran was diagnosed by gene sequencing as was in our patient (Table 2).

Table 2: Molecular genetics - PCR for Thalassaemia.

Mutation	
Mutation (1)	Fr 8-9 (+G)
Mutation (2)	Hb-D (Iran)
Opinion	Compound heterozygous with Hb-D Iran /Fr 8-9(+G) mutation

To our knowledge, we reported the first case of co-inheritance of compound heterozygous Hb D Iran with β thalassaemia trait in Pakistan. The coinheritance of Hb D Iran with β thalassaemia can be found in Pakistani population. Therefore, it is important to investigate patients suspected of haemoglobinopathy in an endemic area who either fail to respond to correction therapy for anaemia or present with clinical features of the disease. In order to prevent and ensure successful control programs of thalassaemia and hemoglobinopathy, it is important to screen young couples for genetic before marriage [16].

Acknowledgement

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Conflict of Interest

Authors declare no conflict of interest.

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