Case Report

Triple Heterozygosity: A Riveting Coinheritance

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Abstract

Medical literature has witnessed various heterozygous combinations between thalassemia and hemoglobin variants posing diagnostic challenges but very few case reports have been reported stating double heterozygosity among hemoglobinopathies themselves, let alone in a combination with thalassemia. We report one such rare presentation of triple heterozygosity on cation exchange-high performance liquid chromatography (CE-HPLC) of beta thalassemia trait coexisting with Hb D Punjab and Hb Q India in an adult female who presented with fever, pain abdomen, vomiting and had a past history of intermittent jaundice and recurrent anemia in childhood as well. A positive family history of patient's father's beta thalassemia trait and patient's mother's Hb D Punjab and Hb Q India helped us clinch the diagnosis in our index case, thus proving family screening to be an inexpensive and rapid way to resolve HPLC patterns.

Keywords:

Beta Thalassemia, Hb D Punjab, Hb Q India, Hemoglobinopathy

Introduction

Hemoglobinopathies, by definition, refer to inherited disorders of hemoglobin (Hb) due to mutations or deletions in globin polypeptide chains. Majority of them arise from single amino acid substitution in globin chains owing to point mutations, whereas in cases where molecular defects affect α, β or δ globin genes result in thalassemia.[1] The prevalence of thalassemia and hemoglobinopathies have been known to vary with geography, estimating about 0.37 per 1,000 fetuses in India. Beta-thalassemia minor, also called as carrier or trait, is the heterozygous state that is usually asymptomatic with mild anemia. Among hemoglobinopathies, a point mutation in beta globin gene in 121 codon at first base (GAA→CAA) with glutamic acid replacing glutamine result in Hb D Punjab (also known as Hb D-Los Angeles) prevalent in Punjab region of North western India (2% of Sikh community) and in countries like Brazil (overall worldwide frequency of 0.2 – 3.0%).[2] The heterozygous composite of Hb

D with Hb S results in severity of sickle cell disease, which is known to cause hemolytic anemia, acute vaso-occlusion, and organ damage due to recurrent erythrocyte sickling. One more uncommon alpha chain variant with a prevalence of 0.4% in Indian subcontinent is Hb Q India resulting from histidine substitution for aspartic acid at codon 64 of the alpha 1-globin gene (AAG→GAG).[3] Literature search has shown coinheritance of Hb Q India with beta thalassemia results in a silent carrier state whereas Hb D Punjab and beta thalassemia co-inheritance presents with mild microcytic and hypochromic anemia.[4] Double heterozygosity of Hb D Punjab and Hb Q India is a very rare compound heterozygous hemoglobinopathy with handful of reported cases to date.[5,6] It is highly uncommon to have a double heterozygous condition, let alone a triple one. Here we report such a rare and unique case of Hb D Punjab, beta thalassemia trait and Hb Q India triple heterozygosity in a 36-years-old female.

Case Report

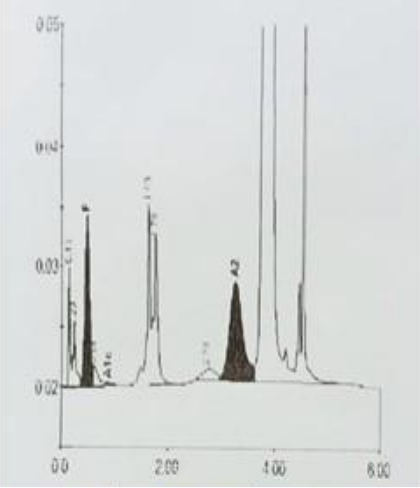
A 36-years-old Indian Punjabi female, resident of Haryana, India presented with chief complaints of fever, pain abdomen and vomiting for six days. She was febrile (100.3'F) on examination with presence of pallor, icterus and hepatosplenomegaly (2 and 4 fingers below costal margin respectively). Her initial investigative workup (Table-1) revealed a low Hb (7.1 gm/dL) along with reduced red blood cell (RBC) indices. Biochemistry reports showed deranged liver function tests and raised C-Reactive proteins (CRP) (29.5 mg/L). Her CRP levels and fever improved on antibiotics however, because of persistent anemia and jaundice, further evaluation was carried out. Written informed consent was obtained from the patient and her family. Ethical clearance was obtained from the institutional ethical committee.

Her past history included episodes of intermittent jaundice and recurrent anemia in childhood with a negative history of any previous blood transfusion. In our setting, ethylene diamine tetra acetic acid (EDTA) anticoagulated blood samples were obtained and run on Sysmex XN-550 automated hematology analyzer which showed low Hb level (6.4 g/dl) with total leucocyte count of 7,580/ul and a reduced platelet count of 99,000/mm3. Leishman-stained peripheral blood film showed a dimorphic, predominantly microcytic hypochromic blood picture. Renal function tests were within normal limit however ultrasound abdomen findings exhibited liver span of 17.4 cm and splenic span of 23.3 cm with grade I fatty liver. Direct Coombs test came out to be negative. In view of persistent anemia and jaundice, further evaluation was done using cation exchange-high performance liquid chromatography (CE-HPLC) (Bio-Rad D10, Bio-Rad laboratories, Hercules, CA, USA) (Figure-1). The findings revealed raised HbA2 levels (6.3%) along with two unknown peaks. One unknown peak (73.6%) was in D window with a retention time (RT) of 3.81 minutes while the other peak (9.1%) was at 4.53 minutes suggesting a possibility of an alpha chain variant. This provisional diagnosis of heterozygous beta thalassemia with Hb D Punjab and an alpha chain variant advocated further evaluation with help of family studies. During her hospital course, Hb levels continued to be low (Table-1).

Patient's father's (65-year, male) investigative reports revealed RBC indices of MCV 68.6 fL, MCH 21.3 pg & MCHC 31.0 g/dL with normal Hb levels (13.6 g/dl), raised RBC count (6.47 million/cumm), Mentzer index of 10.6 and adequate iron stores (serum ferritin 318.1 ng/ml). His Leishman-stained peripheral blood film exhibited microcytic hypochromic blood picture. Figure-2 shows HPLC (VARIANT IITM, β-Thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA) of the patient's father showing a raised HbA2 level (5.6%). His final diagnosis pointed towards beta thalassemia trait. Patient's mother's (58-year, female) investigative reports revealed MCV 98.7 fL, MCH 32.0 pg & MCHC 33.4 g/dL with normal Hb levels (13.1 g/dl), reduced RBC count (3.96 million/cumm) and negative sickling test. Her Leishman-stained blood smear was normocytic normochromic. Figure-3 shows HPLC findings (VARIANT IITM, β-Thalassemia Short Program; Bio-Rad Laboratories, Hercules, CA, USA) of patient's mother with a peak (30.1%) at a RT 4.10 minutes falling in D window, other unknown peak (9.4%) with a RT 4.67

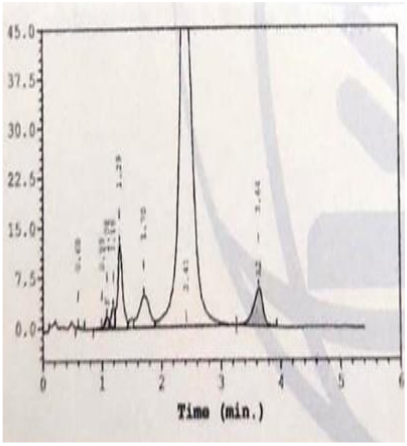
minutes corroborating with Hb Q India and a third peak (7.8%, RT 4.93 minutes) falling in C window correlating with DQ double heterozygous. Hence a final impression of Hb D Punjab, heterozygous with Hb Q India was made. HPLC of both the parents were run on a different machine as compared to our index case.

A positive family history with the HPLC findings of our index case helped us to stumble upon the final diagnosis of Hb D Punjab and Hb Q India coexisting in a setting of beta thalassemia resulting in a triple heterozygous state (Figure-4).



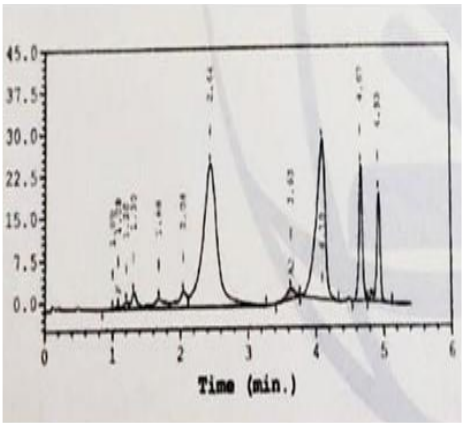
Peak Name	R. time	Height	Area	Area%
Unknown	0.13	9997	18906	0.8
A1a	0.23	5471	20747	0.8
F	0.48	14284	82856	3.4
LA1c/CHb-1	0.61	1646	9682	0.4
A1c	0.87	291	3462	3.2
P3	1.63	15123	65158	2.7
A0	1.76	12544	68085	2.8
Unknown	2.73	861	16055	0.7
A2	3.26	8193	139635	6.3
Unknown	3.81	199401	1806511	73.6
Unknown	4.53	117425	224657	9.1
				Total area: 2455755

Figure 1: Cation exchange HPLC (Bio-Rad D10) of patient shows three peaks- raised HbA2, unknown peaks at 3.81 minutes and 4.53 minutes retention time- pointing towards heterozygous beta thalassemia with Hb D Punjab and alpha chain variant



Peak Name	Area %	Retention Time (min)	Peak area
Unknown	0.1	0.60	1353
Unknown	0.1	0.99	2313
F	0.9	1.08	21484
Unknown	1.3	1.18	28695
P2	6.3	1.29	141048
P3	5.2	1.70	116526
A0	92.0	2.41	1802115
A2	5.6	3.64	122617
		Total area: 2,236,152	

Figure 2: Cation exchange HPLC (Bio-Rad VARIANT II) of patient's father shows raised HbA2 suggestive of beta thalassemia trait



Peak Name	Area %	Retention Time (min)	Peak area
Unknown	0.1	1.00	1143
F	0.3	1.08	6514
Unknown	0.8	1.20	15139
P2	1.9	1.30	36350
P3	2.1	1.68	39888
Unknown	3.0	2.04	57837
A0	42.8	2.44	830746
A2	1.8	3.63	33884
D-Window	30.1	4.10	584148
Unknown	9.4	4.67	181604
C-Window	7.8	4.93	151950
Total area: 1,939,202			

Figure 3: Cation exchange HPLC (Bio-Rad VARIANT II) of patient's mother shows peak in D window (at 4.10 min RT), unknown peak (at 4.67 min RT) corroborating with Hb Q India & a third peak in C Window (at 4.93 min RT) indicating DQ double heterozygosity.

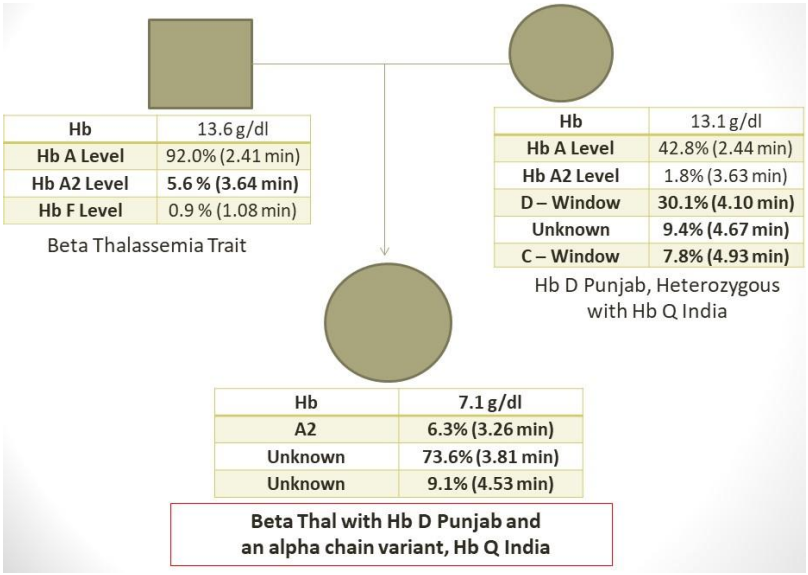


Figure 4: Pedigree of family with beta thalassemia, Hb D Punjab and Hb Q India triple heterozygosity

Table 1: Complete hemogram of novel patient (chronological fashion)

Parameters	**July '22**	**August '22**	**September '22**
Haemoglobin	7.1 gm/dl	6.4 gm/dl	7.0 gm/dl
TLC	5700 /ul	7580 /ul	6760 /ul
DLC	N65 L26 M06 E03	N60 L32 M06 E02	N57 L37 M04 E02
RBC Count	3.21 x 10^3	-	3.43 x 10^3
Hematocrit	20.3%	-	21.6%
MCV	71.7 fL	-	63.0 fL
MCH	21.3 pg	-	2.3 pg
MCHC	29.7 g/dl	-	32.2 g/dl
RDW	29.1%	-	30.3%
Platelets	-	99000	96000

Discussion

In addition to the historical Hb namely HbA, HbS, HbC etc., Hb D was first identified by Itano in 1951 with a similar electrophoretic mobility similar to HbS in alkaline pH however in acidic pH, it resembles HbA in migration. Hb D Punjab is common (2%) among Sikhs of Punjab, India followed by Gujarat (1%) and Iran. Presence of this blood group is also seen sporadically in whites worldwide (0.4%) and in American Indians.[7] The Hb D Punjab usually inherits in heterozygous state with normal HbA, characterizing the heterozygous state with no clinical manifestations. However, the double heterozygous state with Hb S results in moderate to severe sickle cell disease.[8] Hb D Punjab appears as an unknown peak at 3.8 minutes± 0.1 min in Bio-Rad D10 and 4.10 minutes± 0.01 min in Bio-Rad Variant II.

An uncommon alpha chain variant, Hb Q India, shows a similar electrophoretic mobility as Hb S/D at alkaline pH but has a different RT (4.76 mins, Bio-Rad Variant) on HPLC and has been reported among individuals belonging to the Punjabi, Sindhi and Lohana communities. Hb D is suggested to have taken origin in India and then migrated to the world however Hb Q was first reported by Vella et al. in 1958 in a Chinese family.[9] Individuals with Hb Q India are clinically silent, even on its combination with beta thalassemia trait because of the mutation α64 occurring on hemoglobin tetramer's surface, not affecting the properties of hemoglobin molecule. Its diagnosis faces difficulty owing to a normal hematology profile and misinterpretation of Hb Q as HbS/HbD/HbG on alkaline agarose gel electrophoresis because of similar electrophoretic mobility. Hb Q India produces a characteristic sharp narrow unknown peak with a RT of 4.46± 0.01 min on the Bio-Rad D10 and 4.77± 0.01 min on the Bio-Rad Variant II. Beta thalassemia with Hb Q India is incidentally detected during family screening.

Very few case reports of double heterozygosity of Hb D Punjab and Hb Q India have been reported with hybrid HbQ India/ HbD Punjab eluting in HbC window.[5,6] Whereas only one previous case report on triple heterozygosity has been identified on extensive literature search in which Sharma et al. talked about challenges painted by amalgamation of Hb D-Punjab/ Hb Q-India/ Beta Thalassemia trait.[10] Similar to our case, they found a missing Q India peak, despite of patient having an inherited alpha Q-India variant, as there were no β chains available to bind to α (Q India) chain.

This rare case presents an example of the diagnostic difficulties faced during reporting of Hb HPLC and utility of family study in solving such complex cases. An accurate and precise diagnosis of haemoglobinopathies is essential not only for the correct clinical management of the patient but also plays a crucial rule in further counselling of the patient and family, including prenatal counselling.

Both our case and the other similar case report[10] had non-transfusion dependent thalassemia presenting with severe anaemia and splenomegaly, in contrast to the expected phenotype of mild anaemia. Therefore, further studies are warranted to study the effect of coinheritance of this triple heterozygosity - Hb Q India, Hb D Punjab and beta thalassemia on phenotype of patient.

Conclusion

In our extensive literature search, this was the second case report, both in country and worldwide, which depicted triple heterozygous hemoglobinopathies in a single individual advocating the importance of genetics and family screening, especially in a resource constraint setting.

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Competing Interests: None

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