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Case report—unveiling a case of hemoglobin D-Punjab variant with iron deficiency anemia in Sindh (Pakistan)

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Abstract

Hemoglobin D variations are a group of hemoglobinopathies caused by mutations in the genes that control the synthesis of new globin chains. Hemoglobin D-Punjab is the most prevalent but frequently asymptomatic, it can occasionally cause mild to moderate hemolytic anemia, making diagnosis difficult and raising the risk of misdiagnosis. This article discusses a rare instance of a seventeen-year-old male in Sindh, Pakistan with iron deficiency anemia who was later found to have the Punjab variation of the hemoglobin D. The patient had signs of weakness, exhaustion, and shortness of breath, which were initially alleviated by iron supplementation but eventually became refractory. Hemoglobin electrophoresis demonstrated the distinctive hypochromic, microcytic red blood cell shape, and laboratory tests verified the presence of the Hemoglobin D-Punjab feature. The instance emphasizes how crucial it is to distinguish Hemoglobin D-Punjab from other anemias in order to guarantee proper care. This case underscores the importance of recognizing hemoglobin D-Punjab trait, to provide appropriate genetic counseling and ensure the patient's well-being. Increased awareness among healthcare professionals regarding the diverse spectrum of hemoglobinopathies is essential for accurate diagnosis and management.

Keywords

Pakistan, Karachi, iron deficiency anemia, hemoglobinopathies, anemia, Hb D-Punjab

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Introduction

Hemoglobinopathies are hereditary disorders caused by gene mutations responsible for regulating globin chain synthesis. While more than 900 known abnormal HbS are described, only a few recessive traits are highly prevalent and clinically relevant. These are the Hb S, Hb E, Hb C, Hb D-Punjab, and Hb O-Arab variants.[1] Approximately 15 known variations of Hb-D have been reported in the scientific literature. Among these variants, Hb D-Punjab (also known as Hb D-Los Angeles) is the most common. The unique feature of these variants is the presence of the same amino acid substitution (Glu → Gln) at position b121, which gives rise to Hb D-Punjab, Hb D-Los Angeles, Hb D-North Carolina, Hb D-Portugal, and Hb D Chicago.[2] Hemoglobin D Punjab, also known as Hb D-Los Angeles, was first discovered in a family of mixed British and American- Indian origin from the Los Angeles area.[3] It is found in various regions, including India, Pakistan, Iran, Iraq, England, Ireland, Holland, Turkey, and Brazil. It affects both males and females equally. The prevalence of this condition ranges from 1% to 3% of the population, with higher occurrences in northwest India, particularly among the Sikh community in Punjab, as well as in Gujarat.[4] In Pakistan, it has never been reported in Sindh.

Hemoglobin D is considered a rare hemoglobinopathy, often going largely unreported due to its asymptomatic nature. Hemoglobin D-Punjab, a common variant of hemoglobin D, is known to cause a mild disease even in the homozygous state. However, in some cases, individuals with this profile may develop mild to moderate hemolytic anemia. Its diagnosis may be challenging, as it can be easily misdiagnosed or overlooked.[5] Hemoglobin D-Punjab and

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Table 1. Complete blood cell count and iron indices on different follow-ups.

Hematological parameters	Initial presentation	1st follow-up (after 2 weeks)	2nd Follow-up (after 6 weeks)	Reference intervals
TLC	5	2.6	4.2	4–10 × 10^9/L
RBC	5.35	4.52	5.12	3.80–5.80 × 10^{12}/L
Hemoglobin	11.9	10.7	11.5	13–17 g/dl
Hematocrit	38.6	31.8	38.1	40%–52%
MCV	72.1	70.4	74.5	76–96 fl
MCH	22.2	23.8	22.5	27–32 pg
MCHC	30.8	33.3	30.2	31.5–34.5 g/dl
Platelets	215	103	165	150–400 × 10^9/L
Reticulocyte count			2.04	0.5%–2.5%
Neutrophils	45	52	50	40%–80%
Lymphocytes	43	30	40	20%–40%
Monocytes	7	6	9	2%–10%
Eosinophils	5	12	1	1%–6%
ESR	2			0–10 mm/lst hr
TIBC		67		49.0–69.0 µmol/L
Iron		8.8		5–35 µmol/L
Ferritin		9		12–250 ng/ml

ESR: erythrocyte sedimentation rate; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: Red Blood Cells; TIBC: total iron binding capacity; TLC: total leukocyte count.

hemoglobin D-Iran exhibit similar electrophoretic mobility, but they can be differentiated by their retention times on high-performance liquid chromatography (HPLC). On alkaline electrophoresis, Hb D-Punjab migrates similarly to Hb-S. On the other hand, Hb D moves more like Hb A (typical adult hemoglobin) in acidic pH circumstances.[6] The genotype of Hb-D can be detected by DNA amplification and globin chain analysis.[4]

Case presentation

We report a rare case of a 17-year-old male presenting with signs and symptoms of iron deficiency anemia later diagnosed as a case of hemoglobin D variant. The child presented to Emergency/OPD at the tertiary care hospital in Karachi, Pakistan, with complaints of generalized weakness, lack of energy, rapid heartbeat (tachycardia 120 bps), and shortness of breath & increased respiratory rate (32 breaths per second) with activity, which has led to the child sleeping early and not going out with friends to play. The given history dates to 3 months back in time with insidious onset and symptoms have been progressing over time. He had been diagnosed 1 month back before presentation to our institution with iron deficiency anemia for similar symptoms and was advised to have oral iron supplements for 4 weeks. There was an improvement in his symptoms in the early period of treatment, however, later on, the symptoms stopped responding to the tablets.

History dates 6 months back when the child started complaining about generalized weakness, fatigue, and shortness of breath. One month ago, he was brought to the hospital when the symptoms worsened. There was no family history of symptomatic anemia, repeated blood transfusions, and no personal history of recurrent fever, jaundice, prior blood transfusions, pica, or severe weight loss.

A general physical examination of the patient concluded a pale-looking and malnourished with a low body mass index (BMI) (17.5), ill-appearing child vitally stable, with a heart rate of 120 beats/min. There was scleral icterus, pale pink coloring on the inside of the lower eyelids, pale skin, and cold hands and feet. The child had no edema, peripheral lymphadenopathy, or digital clubbing. On Cardiovascular examination, the patient had normal heart sounds with a systolic ejection murmur II/VI heard at the left sternal border. There was a soft and non-tender abdomen with no organomegaly on abdominal examination. There was no other significant finding on all other systemic examinations.

Laboratory investigations (Table 1) from 1 month back showed hemoglobin of 11.9 g/dl, red blood cell count of 5.35, mean corpuscular volume of 72.1 fl, mean corpuscular hemoglobin concentration of 30.8 g/dl, serum iron 8.8 µmol/L, total iron binding capacity 67 µmol/L, and serum ferritin 9 ng/ml. Also revealed normal erythrocyte sedimentation rate (ESR), fasting, and random blood glucose levels. On the peripheral smear, there was hypochromia, microcytosis, anisocytosis, and poikilocytosis. On Hemoglobin Electrophoresis, Hb A2 was 2.9 (Table 3), the HPLC showed peaks of Hb-A, and an unknown band was seen at 3.93 retention time (Figure 1 and Table 2). Findings were suggestive of the Hb D-Punjab trait.

As the patient was presenting with mild symptoms and according to the lab results, was nontransfusion dependent, he was treated in an outpatient department with iron

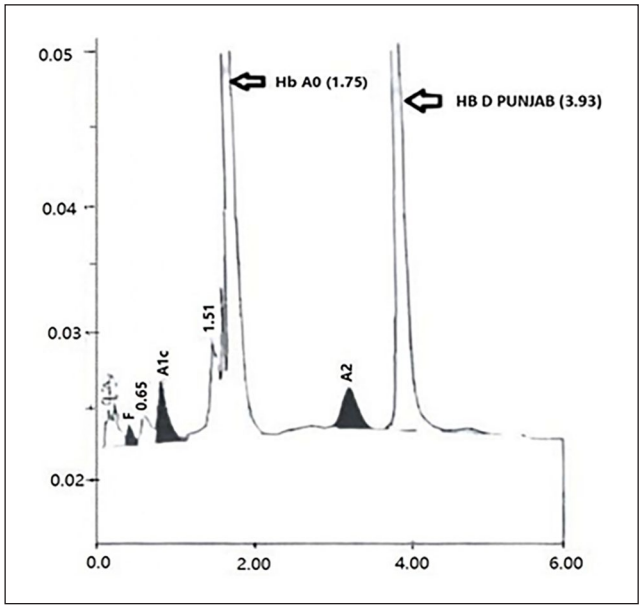

Figure 1. Chromatogram of glycated hemoglobin on high-performance liquid chromatography performed on Bio-Rad machine showing a peak at retention of 3.93 which is suggestive of Hb D-Punjab trait; *X* axis: Retention time (min), *Y* axis: Volts.

Table 2. Peak table; The values obtained from high-performance liquid chromatography; Figure 1 was plotted by the software according to these values.

Peak	R. time	Height	Area	Area%
A1a	0.2	2726	14836	0.9
A1b	0.28	3227	13151	0.8
F	0.46	1438	9231	<0.8
LA1c/CHB-1	0.65	2087	17322	1
A1c	0.86	4280	40381	5.4
P3	1.51	7197	63131	3.7
A0	1.75	21901	935036	55.2
A2	3.22	2891	43906	2.9
Unknown	3.93	98300	556796	32.9
Total area	1,693,790			

Hb: Hemglobin; R. time: Retention Time.

supplements (containing Iron Bisglycinate 10 mg per day), folic acid (300 mcg per day), iron-enriched food and with appropriate counseling, as there was noncompliance history from the mother due to limited awareness in adhering to the prescribed regimen and not stopping consuming when they notice improvement in the patient's health, therefore, close follow-up was planned. On a follow-up visit, repeated laboratory tests revealed improved blood results, hemoglobin levels of 12.5 g/dl, mean corpuscular volume 77.5 fl, mean corpuscular hemoglobin concentration 33.2 g/dl, serum iron 10 µmol/L. On current follow-up, the patient had alleviated symptoms as compared to the previous visit, decreased generalized weakness, shortness of breath now only on exertion as compared to at rest previously, and normal heartbeat and respiratory rate which were elevated on prior visitation.

Table 3. Concentration of each type of hemoglobin acquired from high-performance liquid chromatography.

Concentration	%
F	<0.8
A1c	5.4
A2	2.9

Discussion

In 1951, Itano uncovered the existence of Hb-D in a multiracial British and American- Indian family living in Los Angeles. It is predominantly found in the Punjab region, Northwest India, and Pakistan, with only a 2.0% rate of occurrence.[4] Hb D-Punjab disease presents in these six types: 1. Hemoglobin D trait (Hb-AD); 2. Hemoglobin D disease (Hb-DD); 3. Hemoglobin D–thalassemia (thalassemia trait, AD trait, or DD homozygous); 4. Double heterozygous S and D (Hb-SD); 5. Hemoglobin D Iran disease 6. Double heterozygous E and D (Hb-ED).

The heterozygous state (Hb-AD), homozygous (Hb-DD), Hb-D Iran heterozygous, and double heterozygosity for Hb-E and Hb-D are all clinically benign conditions.[4] Hb D Homozygous states are less frequent than Hb D β thalassemia and it commonly presents with mild hemolytic anemia. Whether Hb D β thalassemia will be symptomatic is influenced by the inheritance pattern of β thalassemia; β^0 $\beta+$ will present with no significant symptoms that are of mild to moderate hemolytic anemia, whereas coinheritance of β^0 β^0 and Hb D have a destructive impact on health with moderate severity chronic hemolytic anemia, accompanied by splenomegaly.[4,7] Double Hb-SD is the most severe form of Hb D-Punjab. The consequences of the disease are more stringent than only heterozygous Hb S; on the other hand, they have slightly milder symptoms than homozygous Hb SS. Higher chances of red cell lysis in Hb-SD make splenomegaly and vaso-occlusion more prevalent in Hb-SD than in homozygous Hb SS.[2] Further explanation is provided in Table 4. Previous literature indicates that the Hb D trait rarely manifests clinically and is mostly discovered incidentally when another pathological or physiological condition puts the body's hemostasis in overdrive and causes anemia, in coherence, Singh et al.[2] reports 13 symptomatic cases of the Hb D trait with the coexistence of hereditary spherocytosis, anemia due to blood loss, iron deficiency anemia, low Hb levels associated with Gaucher disease and one under the suspicion of alpha thalassemia, similarly Huit et al.[7] also set forth an instance of Hb D trait and iron deficiency anemia in pregnancy (a physiological condition). Our case presented mainly with mild symptoms: generalized weakness, lack of

Table 4. Characteristics of different types of anemia with Hb-D.

Type of anemia	Characteristics	Electrophoresis	Differential diagnosis	Associations with hematological diseases	Treatment	Prognosis
Sickle cell anemia	One of the beta-globin subunits in hemoglobin is replaced with hemoglobin S	HbS on electrophoresis	Thalassemia, hemoglobin C disease	Increased risk of infection, vaso-occlusive crises	Hydroxyurea, Blood transfusions, Folic acid	Variable, depends on complications and severity
Hb D-Punjab thalassemia	A point mutation in the beta-globin gene (*HBB*) in the first base of the 121 codon (GAA→CAA)	HbA, HbD, and HbF on electrophoresis. HbD was seen at 3.93 min.	Beta-thalassemia	Thalassemia major, beta-thalassemia intermedia	Blood transfusions, Iron chelation	Variable, depends on thalassemia severity
Hb D-Los Angeles	A glutamic acid to glutamine substitution at codon 121 with electrophoretic mobility at alkaline pH is similar to HbS	HbA and HbD on electrophoresis detected at 4.1–4.3 min.	Hemoglobinopathies	None specific	Supportive care, Blood transfusions	Variable, depends on complications

energy, rapid heartbeat, and shortness of breath with activity, which are also typical iron deficiency anemia symptoms leading to the initial diagnosis; nevertheless, persistently deranged investigations evoking further exploration through Hb electrophoresis and HPLC tests. Our patient also experienced two uncommon symptoms, which usually correspond to shallow hemoglobin values, scleral icterus, and hemic murmur. There could be an accompanying mechanism of mildly low Hb with Hb D-Punjab for which there is no explanation in the literature, although it has been reported previously.[6,8]

While molecular studies are the best for diagnosing hemoglobinopathies, in this region, they often use more affordable methods like HPLC and Capillary Electrophoresis (CE) due to limited funds.[9] HPLC separates and identifies specific hemoglobin percentages in blood samples using ion exchange, while CE uses electrokinetic separation to detect hemoglobin fractions.[10] Both methods rely on the electrophoretic mobility difference of hemoglobin variants. It's important to mention that molecular studies, although recommended as the final step, are not always done in most cases. In a typical individual, CE reveals that hemoglobin A levels range between 96% and 98%, with hemoglobin F below 1% and hemoglobin A2 less than 2%,[9] these percentages are obtained from peak area, which denotes the amount of each type of Hb contributing to the total Hb, in our study it is mentioned under the heading area % in Table 2. On HPLC, Hb D-Punjab characteristically separates at an unknown window with a peak at the retention time of approximately 3.8 ± 0.1 min (Hb D window (RT, 3.8–4.3 min)), distinguishing it from the Hb-S peak (Hb S window = RT = 4.3–4.7 min).[11] Hb D in the heterozygous state amounts to 30%–45% of the total Hb, normal Hb F percentage, although Hb A2 values are on the lower side of average values or slightly increased (0.9%–2.5%). On the contrary, in Hb D Homozygous Hb D contributes to more than 90% of total hemoglobin; however, Hb F and A2 values (percentages) are not affected.[4] Even Though Hb D β thalassemia CE-HPLC exhibits a similar retention time of the peak to Hb-AD and Hb-DD, in contrast to them, Hb-D accounts for 70%–90% of total Hb, diminished Hb A0's peak, in addition, Hb A2 and Hb F (3%–20%) peaks are raised.[4,7] CE-HPLC for Hb-SD displays two additional peaks at the retention time of Hb D and Hb S window, furthermore, Hb S and Hb D contribute to 30%–35% and 40%–45% of the Total pool of hemoglobin in each Hb-SD patient, respectively.[11] Hb F peak is considerably increased than usual in Hb-SD, hence contributing more to the total Hb as compared to normal. Hb D-Iran and Hb-ED illustrate similar peaks in the A2 window (3.39 min RT), thus alkaline electrophoresis is utilized to differentiate them as Hb D Iran electrophoretic mobility coincides with Hb A2, and Hb E accompanies Hb S and Hb D-Punjab.[12] In our case, HPLC elicited an unknown peak at the retention time of 3.93 (Figure 1 and Table 2), pointing toward the diagnosis of Hb D-Punjab, furthermore, of the total hemoglobin, Hb D-Punjab accounted for only 32.9% (Table 2) which is highly suggestive of heterozygous state (Hb-AD).

Rbc indices and peripheral smear of Hb-AD are usually normal, however, concomitant pathological conditions can alter them.[4] Our case laboratory investigations showed microcytic and hypochromic RBCs, low Hb levels, anisopoikilocytosis on peripheral smear, and low ferritin levels, alongside the results of HPLC prompting the diagnosis of iron deficiency anemia with Hb D-Punjab trait, similar findings are also reported by, Huit et al.[7] in their pregnant iron deficient Hb-AD patient and Pandey et al.[8] in their six symptomatic Hb D trait patients. Hb D β thalassemia exhibits identical rbc indices to Hb-AD trait, RBCs in high number, smaller in size, and less red than usual, however, as explained above they are differentiated on the basis of HPLC.[4]

Conservative management was opted, considering that these symptoms don't cause life impairment; hence, he was advised to avoid strenuous exercises, and rigorous counseling related to marriage was done although his parents were not investigated. Premarital counseling includes the fact that there is a 25% (one in four) probability an offspring with hemoglobin D/β^0 thalassemia (Dβ^0) or double Hb-SD is conceived when one parent has the β^0 thalassemia trait or sickle cell trait, respectively, when the other parent has the Hb D trait.[4,6] On this account, the spouse must undergo these tests before planning on procreating, as both conditions hinder the quality of life due to severe health conditions.

Dash et al.[13] have also linked hematological malignancies with Hb-D in Punjab and reported two cases. A 27-year-old male issue of heterozygous Hb D-Punjab diagnosed with acute myeloid leukemia (M3 FAB) and a 14-year-old boy, homozygous for Hb D-Punjab, discovered to have Hodgkin disease, lymphocytic predominance type, in stage IVB. Kumaresan K also describes an occurrence of adrenal myelolipoma in a 24-year-old who was double heterozygous for Hb D-Punjab and B thalassemia.[14]

Pakistan, being a third-world country where primary healthcare is not readily available, makes this clinically silent condition not very feasible to detect. Even when it is detected, proper marital and family counseling is not performed by the clinicians, as they are not very well versed in it. This case is sporadic, with its asymptomatic nature and its association with malignancies and severe conditions when inherited along with $\beta^0\beta^0$ Thalassemia and Hb S, makes genetic counseling of the whole family mandatory, and regular check-ups along with blood work should be advised even if they notice a slight change in their health.

Conclusions

This article emphasizes the value of thorough hematological assessments in cases of anemia, considering Hb-D variations as part of the differential diagnosis. Individuals with hemoglobin D-Punjab and other related variations may benefit from early detection, efficient care, and better results with the help of doctors and laboratory professionals working together using new diagnostic technology. Ongoing research and education are necessary to comprehend hemoglobinopathies better and raise the standard of care.

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I.J.: have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; M.O.A.: been involved in drafting the manuscript or revising it critically for important intellectual content; F.B.F.: given final approval of the version to be published; S.A.J. and S.A.M.S.: agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Data availability

The authors confirm that the data supporting the findings of this study are available within the case report.

Declaration of conflicting interests

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Ethics approval

Our institution does not require ethical approval for reporting individual cases or case series.

Informed consent

Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

Human subjects

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