Trace element status in a rare case of hemoglobin D Punjab trait

Sudha K., Prabhakaran N.

[1]Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, India

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Corresponding author: **Sudha K**. Email: sudha.k@manipal.edu

ABSTRACT

Hemoglobin D Punjab is a hemoglobin variant that occurs mainly in northwest India with prevalence rate of 1%. Homozygous form is very rare and clinically more serious than heterozygous HbD. Here we present a case of HbD trait with mild anemia and slightly altered red cell indices in a 31year old female from South India. Decreased plasma trace elements (iron, copper, zinc) and altered metalloproteinsare the novel findings, emphasizing the need for periodic assessment and prompt supplementation of micronutrients to prevent red cell damage and related complications in hemoglobinopathies.

Keywords: Hemoglobin D Punjab; hemoglobin variants; trace elements.

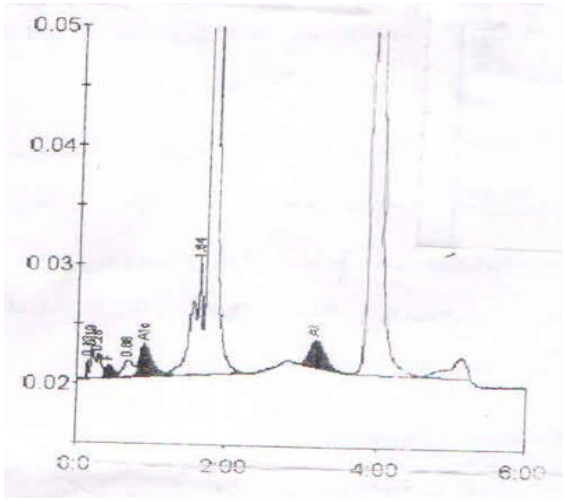
INTRODUCTION

Hemoglobin D Punjab is a rare hemoglobinopathy with β 121(GAA →CAA) mutation leading to substitution of glutamic acid by glutamine (1). It is asymptomatic with no clinical and hematological alterations in heterozygous state, but a subset of patients may manifest with chronic mild hemolytic anemia. Patients will have HbD, small percentage of HbA depending on the type (β0, β+) mutations, normal or slightly increased HbA2 and HbF. Homozygous HbD disease is a more serious condition with splenomegaly and moderate hemolytic anemia(2). In addition to iron, trace elements copper and zinc also play a crucial role in heme biosynthesis. Further, being a part of erythrocyte membrane SOD, they may increase the RBC life span (3).We hypothesize that trace element deficiency may add to the clinical complications in hemoglobinopathies. Hence, plasma trace elements and metalloproteins were analyzed in Hb D patient.

Case: A 31year old female, mother of six months old child complains of generalized weakness, fatigue and inability to do her routine work from previous few months. General practitioner found her to be pale with low hemoglobin, suspected iron deficiency anemia and prescribed iron supplements. When her condition did not show any improvement even after treatment for 6 months, she was referred to a hematologist. On clinical examination, the patient had no splenomegaly, blood smear showed anisopoikilocytosis with microcytic hypochromic red cells. Red cell indices revealed the following: Hemoglobin 11.5g%, RBC count 3.9 x 10^6/µL,PCV34.9%,reticulocytes 0.8%, MCV 88.2fL, MCHC32.9 g%, MCH 29 pg, RDW 15.1%, WBC count 6600 cells/cumm, platelet count 345000 cells/cumm. Further LDH levels were normal (192U/L) and sickling test was negative.

Even with oral iron supplements her hemoglobin and ferritin levels were lower, hence hemoglobin variant analysis was recommended, to rule out any hemoglobinopathies. Hb analysis by HPLC quantified HbA at 53.9%, HbA2 3.5%, Hb F< 0.8%.The chromatogram showed an additional peak with retention time of 3.93mins corresponding to Hb D Punjab with 40.7%of the

total hemoglobin (Fig. 1).The chromatogram confirmed a rare case of Hb D Punjab trait.



Peak table – ID: 07767

Peak	R time	Height	Area	Area%
Unknown	0.13	1564	2491	0.2
A1a	0.19	2399	9935	0.7
A1b	0.28	2036	8667	0.6
F	0.42	1113	7979	< 0.8*
LA1c/CHb-1	0.68	1416	13617	0.9
A1C	0.91	2683	30531	5.2
Unknown	1.64	9943	86396	6.0
A0	1.75	145730	667884	46.5
A2	3.17	2237	31665	3.5
Unknown	3.93	76076	578611	40.7
Total Area			1437776	

Concentration	%
F	< 0.8*
A1 C	5.2
A2	3.5

Fig. 1: Chromatographic profile of HbD Punjab trait

Since plasma iron and ferritin were towards lower side of the normal, we estimated other trace elements copper and zinc that had a role in heme metabolism and related metallo- proteins (Table 1). Both copper and zinc levels were lower in the patient studied and SOD was higher than the normal range.

Table 1: Plasma trace elements and metalloproteins in HbD Punjab

Parameters	Normal values	HbD Punjab
Iron (µg/dL)	37 -145	60.63
Copper(µg/dL)	83 - 109	83.2
Zinc (ppm)	0.78 – 0.88	0.5
Ferritin (ng/mL)	13 - 150	19.54
Ceruloplasmin (mg/dL)	20 - 40	28.4
SOD (U/mL)	2 – 3.4	3.6

DISCUSSION

Mutations in genes coding for hemoglobin chains is seen in about 7% of worldwide population, that can either decrease the rate of globin chain synthesis as in case of thalassemia's or modify amino acid make up generating hemoglobin variants. Hb S, C, D are some of the variants produced as a consequence of point mutation (1). Different to other hemoglobinopathies, Hb D is still poorly studied though it is prevalent in Punjab and north western India with an estimated frequency of 1%.Hb D occurs in 4 forms: HbD trait, HbD thalassemia, HbSD disease and HbD disease. HbD disease usually presents as mild to moderate hemolytic anemia with moderate splenomegaly. Adachi et al., (4) published a study on HbSD with severe clinical symptoms and opined that HbD favors polymerization of HbS, which was later confirmed by Patel et al., (5). HbD trait patients are mostly asymptomatic with no changes in the blood indices but the case studied by us had mild anemia and minor hematological

variations showing anisopoikilocytosis. In one of the earlier studies done on 30 HbD trait patients, 24 patients were asymptomatic, and 6 patients showed clinical symptoms and hematological alterations similar to the findings in our patient(6).Trace elements viz., copper and zinc play a major role in erythrocyte function by being an integral part of metalloproteins like SOD and ceruloplasmin. A chronic imbalance in oxidants within RBC makes subjects with haemoglobinopathies more prone to oxidative damage, acceleratingred cell turnover (7).Erythrocytes act as a sink of superoxide ions and a rise in plasma SOD indicates oxidant surge in the patient studied. Ceruloplasmin oxidizes iron and incorporates it to Apo transferrin and transports it to bone marrow for erythropoiesis. Several researchers have also reported zinc and copper deficiency in patients with hemoglobinopathies (8). Hence, trace element deficiency might have added to the morbidity in Hb D Trait studied.

The study highlights the need for periodic assessment and prompt administration of micronutrients to reduce the extent of oxidative damage to erythrocytes and related complications in hemoglobinopathies.

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