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CASE REPORT

HAEMOGLOBIN H DISEASE: A DISEASE LOST IN THE CROWD!

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ABSTRACT

Hb H disease is the most severe form of α-thalassemia caused by deletions of variable length or point mutations in 3 α-globin genes. The prevalence of alpha thalassaemia in India varies from one sub-geographical area to another. Sporadic cases have been reported from India. It generally presents as microcytic, hypochromic anemia and go unnoticed unless there occurs an acute hemolytic crisis during infection, pregnancy or following use of oxidative drugs. Most often, they are under-diagnosed or misdiagnosed as iron deficiency anemia. We report a case of 18 year old boy who presented with fever with severe anemia and severe splenomegaly. Complete blood count (CBC) showed decrease in Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and increase in Red Cell Distribution Width (RDW) by automated cell counter. Peripheral smear showed microcytic hypochromic Red Blood Corpuscles (RBCs) with severe anisopoikilocytosis. Supravital staining of peripheral blood showed HbH inclusions in RBCs. Hemoglobin electrophoresis showed decreased HbA_2 fraction with HbH. The case is hence reported to highlight the importance of simple peripheral smear examination, and in suspected cases, the need to do hemoglobin electrophoresis along with supravital staining of the erythrocytes or else the case may be misdiagnosed and treated as iron deficiency. It is also important to emphasize the importance of early diagnosis to facilitate implementation of proper preventive health care measures and prompt treatment of potentially serious haemolytic crisis.

INTRODUCTION

Thalassemia is the most common single-gene haemoglobinopathy in the world. More than 50% of the world population has a clinically silent form of α-thalassemia. It is more prevalent in South East Asia, Middle East and Mediterranean and in Indian subcontinent because of high carrier frequency of (--SEA) type of α-thalassemia deletion gene [(1)]. The clinical phenotypes of most individual with Hb H disease are very mild and may not be noticed during the entire life unless a routine full blood count is done or when there is an acute hemolytic crisis. Most alpha thalassemia cases in India have been reported among tribal population [(2)]. We report a case of HbH disease in a 21 year old tribal adult who was here before undiagnosed to have thalassemia.

Case Report

A 21 years old male, student by occupation, was admitted for fever with chills 4 days prior to hospital admission. Fever was associated with generalised body aches and extreme weakness. There were no other associated symptoms like vomiting, loose stools, joint pains, burning sensation while passing urine, headache and cough. There was history of receiving blood transfusion 7 years back during hospital admission for malarial fever. At that time, he was also told to have enlarged spleen. Since then he had noticed gradual enlargement in the size of the abdominal lump without pain. There was no past history of any significant medical ailment. He had not used any herbal or ayurvedic preparations and did not have any addictions. He was the only child in his family.

His father had expired few years back due to some illness, which he was not aware of. He was not on regular blood transfusion but was transfused 2 units of blood before his death. His mother had not received blood transfusion so far. On examination, he was coherent, had severe pallor, mild icterus, no lymph nodes, no skeletal deformities and haemolytic faces. His secondary sexual development was normal. He was hemodynamically stable. He had venous hum and a haemic murmur on auscultation. Examination of abdomen revealed mild, firm, non tender hepatomegaly and moderate, non tender spleen up to the umbilicus **(figure 1)**. Examination of other systems was normal.

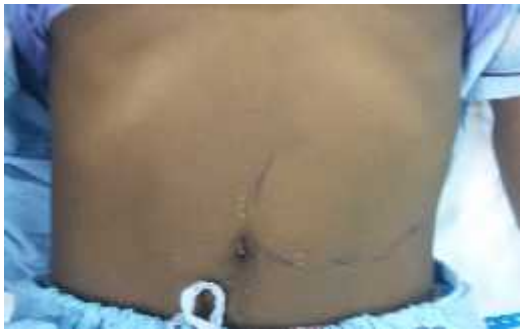

Figure 1 Abdomen showing moderate splenomegaly

His biochemical parameters showed Random blood sugar (RBS) 124 mg/dl, serum creatinine 1.0 mg/dl, serum sodium 132 mmol/L, serum potassium 4.3 mmol/L, total serum bilirubin 2.71 mg/dl, indirect bilirubin 1.72 mg/dl, ALT 160U/L, AST 246U/L, ALP 69.2U/L, serum proteins 6.48g/dl, serum albumin 3.75g/dl, serum globulin 2.73g/dl, INR 1.13, serum LDH 896.7U/L, serum iron 186.5 mcg/dl, serum ferritin 10,424 ng/ml, serum B_{12} 234 ng/L, CPK 124U/L and CRP 0.78 mg/dl. His serum was brownish in colour. The haematological parameters were as tabulated below **(table 1)**.

Table 1 Haematological parameters on admission

Parameters	Values	Reference Range
Haemoglobin (g/dl)	5.1	11.5 – 16.5
Total leucocyte count (/cu mm)	3,400	4,000 – 11,000
Neutrophils (%)	51	60 – 70
Lymphocytes (%)	40	30 – 40
Monocytes (%)	5	2 – 8
RBC count (mil/cu mm)	2.72	4.5 – 5.5
Haemtocrit (HCT) (%)	17.6	38 – 50
MCV (fl)	64.6	76 – 96
MCH (pg)	18.4	38 – 50
MCHC (pg)	28.4	27 - 32
RDW (CV) (%)	18.8	31.5 – 34.5
Platelet count (/cu mm)	56,000	1,50,000 – 4,10,000
PDW (fq)	20.1	-
Reticuloctye count (%)	1.5	1% of RBCs

Peripheral blood smear showed microcytic, hypochromic anaemia with anisocytosis and poikilocytosis. Sickle red cells and other abnormal cells were not found. Malarial parasites were not seen **(figure 2)**.

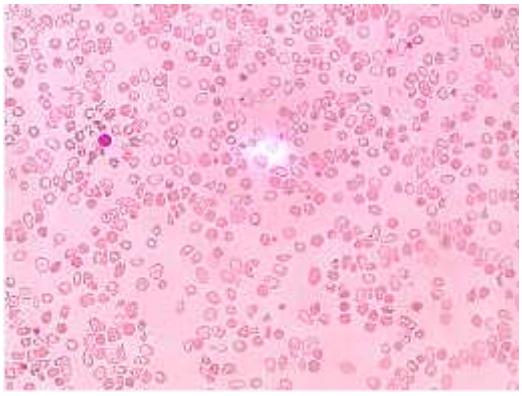

Figure 2 Microcytic hypochromic peripheral smear with anisopoikilocytosis

Direct and indirect Coomb's test were negative. Osmotic fragility was normal. Haemoglobin electrophoresis done by capillary zone method showed HbA 90.3%, HbH 8.3% and HbA2 1.4% (range: 1.5 to 2.5%) **(figure 3)**.

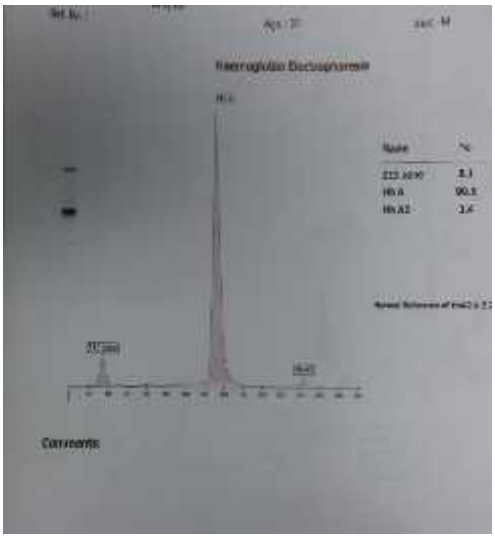

Figure 3 Capillary zone haemoglobin electrophoresis showing HbH

Supravital staining with 1% brilliant cresyl blue of peripheral blood showed HbH inclusions **(figure 4)**. Bone marrow aspirate was hypercellular and showed marked erythroid hyperplasia, normal myeloid maturation, adequate megakayocytes **(figure 5)** and an iron of grade 3+ on Perls' stain. No parasites were seen. Urine microscopic examination was normal. Cultures of bone marrow, blood and urine were sterile. Antibodies to HCV and surface antigen of HBV were not found. Repeat serum ferritin was 2,384 ng/ml and serum LDH was 245 U/L. A diagnosis of alpha thalassemia (HbH disease) with infection induced hemolytic anaemia secondary haemochromatosis was made.

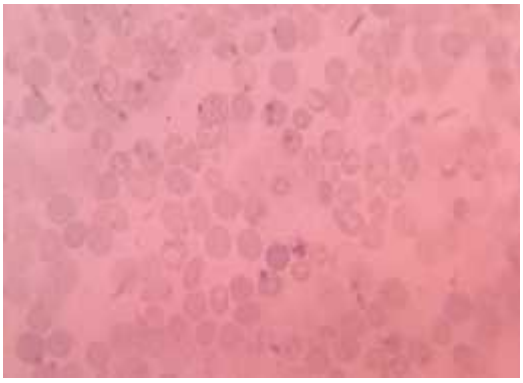

Figure 4 Supravital staining showing the Heinz bodies

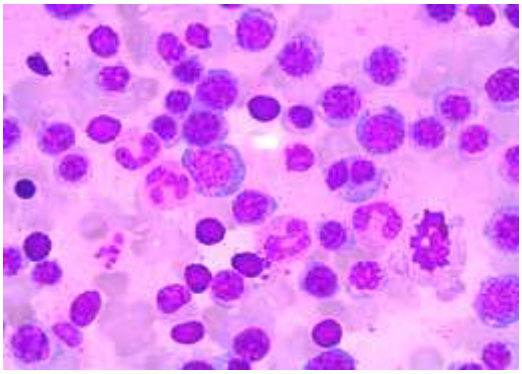

Figure 5 Bone marrow showing erythroid hyperplasia

He was advised to undergo gene sequencing test to identify the mutation. However, due to financial constraints, he refused.

He was transfused 4 units of packed RBCs, given folate 5 mg once a day oral supplement and started on oral iron chelating agent – deferiprone 1g twice a day. He was also asked to avoid iron supplements in future. He did not develop fever after hospital admission and was discharged on the 6th day.

DISCUSSION

Alpha (α) thalassaemia are the most common inherited disorders of hemoglobin (Hb) synthesis caused due to deletions of various lengths or point (non-deletion) mutations affecting one or more α-globin genes leading to decreased or absent α-globin chain synthesis [1,3]. The α-globin gene cluster is located on chromosome 16(16p13.3). α globin chains are structurally normal though they are reduced in quantity. Haemoglobin H (Hb-H) disease is a heterozygous state that results from the loss of three α-globin genes (- α/- -) or interaction of deletion and a non-deletional defects (- -/$\alpha^T\alpha$) [4]. A varied prevalence of α-thalassaemia ranging from 1% to 18% has been reported in the general Indian population [5].

Hb H disease is a special variant of α-thalassemia that is more prevalent in South East Asia, Middle East, Africa, Mediterranean region and in the Indian subcontinent. In India, there have been sporadic reports of alpha thalassaemia [1,4]. Some cases of Hb-H disease have been reported from the tribal population of West Bengal, Bihar, Karnataka, Manipur and from Punjab in Northern India [4]. In India, deletional Hb H disease is common. In a study of molecular diversity of 8 cases of haemoglobin H disease from India by Nadkarni *et al* (2010), 88% of the chromosomes had deletional alleles ($--^{SA}$, $--^{SEA}$). The $-\alpha^{3.7}$ deletion was the most common defect encountered[3].

The clinical severity of HbH disease depends on the type of mutation. The most common form is the deletion type, which causes a milder form of HbH disease [5,6]. These patients have mild anaemia in the baseline state and may require intermittent transfusion therapy especially during intercurrent illness. Chronic transfusion therapy is very uncommonly required. However, patients with non-deletional mutation, constant spring mutation and α_2 gene affection have splenomegaly, mild skeletal features of haemolytic anaemia, moderate to severe anaemia and require more regular transfusion and ultimately splenectomy [6,9]. Hb-H disease is compatible with survival into adulthood.

Hemoglobin H has extremely high affinity for oxygen and therefore is not useful for oxygen exchange, leading to tissue hypoxia disproportionate to level of haemoglobin. It is prone to oxidation, leading to formation of intracellular inclusions [7]. The instability of HbH is a major cause of anemia, as precipitates of oxidized HbH form in older red cells, which are then removed by splenic macrophages leading to hemolysis [8].

When the level of α globulin gene synthesis falls below 70% of normal in adult life, the excess β globulin chains form tetramers of Hb-H (β_4) in the red blood cell (RBC) called Heinz bodies [1,6,8]. The typical inclusion-body cells have a golf-ball like appearance. They bind with band 3 protein in RBC membrane and cause local oxidative damage, membrane dysfunction, shortened red cell survival mostly of mature RBC and a lesser extent to erythroid precursors. Conditions like infections, fever, pregnancy and intake of certain oxidant drugs increase the Hb-H inclusions leading to acute hemolytic crisis and precipitous drop in haemoglobin. All affected individuals have a variable degree of anemia, (haemoglobin 7 to 10%), decreased MCV (60 to 70fl) and MCH (~18pg) and normal or slightly reduced level of HbA_2 [6,9]. The amount of Hb H varies from 1-40% depending upon the severity of the case. The diagnosis is established by haemoglogin electrophoresis (capillary zone electrophoresis, agarose gel electrophoresis) where Hb-H separates out with other haemoglobins. The diagnosis is further established by incubation of peripheral erythocytes for 1 - 3 hours at 37°C in presence of supravital dye, which induces precipitation of HbH as inclusion bodies (Heinz bodies) [8]. As a rule, at least 10% of the cells develop Hb-H inclusions. Molecular analysis (gene sequencing/DNA sequencing) is done to find out the nature of genetic abnormality.

The important differential diagnosis of microcytic anaemia in India is iron deficiency anaemia (IDA) which also presents with decreased MCV, MCH, MCHC and increase in RDW (red cell distribution width) and reticulocyte percent. No RBC indices can reliably indicate the presence of α thalassemia in the subjects with or without IDA [2]. The discrimination between the microcytosis caused by the two conditions is clinically difficult. In our case serum iron and ferritin were both increased and bone marrow examination also increased iron stores thus iron deficiency was ruled out. As ferritin is also an acute phase reactant, the initial value was very high due to acute infection while the second value was lower though still more than two thousand. 70% to 75% of adult patients with Hb H disease have raised serum ferritin levels due to iron overload which is not related to previous history of transfusions or iron supplementation [6]. It is probably due to increased iron absorption, secondary to enhanced erythropoiesis as a result of hemolysis and anemia. Iron overload is also seen in older patients (>45 years) and those on regular transfusion. He had mild hepatomegaly with hepatic dysfunction due to iron overload as seen by elevated AST/ALT ratio >1.5. In contrast, he had severe splenomegaly (20 cm) with hypersplenism which caused pancytopenia. Hepatosplenomegaly are common in adults with Hb-H disease. The incidence of splenomegaly reported in literature varies from 47% to 80% while that of hepatomegaly varies from 15% to 70% [6].

The other causes of microcytic anaemias include haemoglobinopathies, lead poisoning, sideroblastic anaemia, and Haemoglobin E disease. Very high ferritin, increased bone marrow iron stores did raise the possibility of sideroblastic anaemia, but the absence of the sideroblasts in the marrow ruled out the diagnosis. The diagnosis was confirmed by capillary haemoglobin electrophoresis which showed increased haemoglobin H fraction with decreased haemoglobin A_2 and demonstrating Hb-H inclusions in the erythrocytes. Other conditions where inclusions of HbH are found are erythroleukemia and myelodysplasia which are diagnosed by bone marrow examination [1].

Because Hb H disease is uncommon in India and the majority of patients have mild to moderate thalassemia intermedia–like manifestations, current antenatal thalassemia screening program does not usually screen couples for risk of conceiving fetus with Hb H disease. When one parent carries α^o thalassaemia (--/αα) gene and the other carries an α^+ thalassaemia (-α/αα) gene the risk of their offspring having Hb-H disease is 1:4 (25%) while the risk of HbH disease is 1:2 (50%) if the carrier of α^+ thalassaemia is a homozygote. Therefore, in these cases prenatal diagnosis should be done by DNA analysis obtained by chorionic villous sampling at 9 to 12 weeks and timely genetic counselling should be done to

prevent the birth of thalassaemic child [1]. Genotyping α globin may be useful for prognosis of HbH disease, non deletional forms being more severe than deletional forms.

The treatment for Hb-H disease is primarily preventive and supportive in nature. With haemolysis and increased erythropoiesis, folic acid supplement is recommended. Avoiding oxidative medications, iron supplements unless iron deficiency is documented, prompt treatment of infections, and alertness to the possibility of either hypersplenism or regenerative anaemia are indicated [6,8,9]. Adults should be periodically monitored for possible iron overload and related organ dysfunction. When indicated, iron chelation therapy should be instituted. Splenectomy should be done if anemia is severe or transfusion requirement develops. Stem cell transplant and gene therapy are under research trial and may offer permanent cure in near future.

CONCLUSION

HbH disease is an under-diagnosed entity in the Indian subcontinent. It presents as microcytic, hypochromic anaemia, the severity of which depends on the type of mutations. All patients with microcytic anaemia should be careful evaluated for thalassemia, after ruling out iron deficiency anaemia or if they do not respond to adequate iron supplementation. Careful study of the peripheral smear preparation and in suspicious cases doing haemoglobin electrophoresis and staining with supravital dyes would help in diagnosing these cases. Lack of awareness if this condition may lead to wrong diagnosis of iron deficiency anaemia, leading to inadvertent iron overload in these patients.

References

1. Sindhu R, Behera SK, Mishra DP. Hemoglobin H Disease. A rare case report and its diagnostic challenge. *Annals of Applied Bio-Sciences*.2015; 2:C22-4.
2. Sharma M, Pandey S, Ranjan R, Seth T,Saxena R. Prevalence of Alpha Thalassemia in Microcytic Anemia: a Tertiary Care Experience from North India. *Mediterr J Hematol Infect Dis* 2015, 7(1): e2015004, DOI: http://dx.doi.org/10.4084/MJHID.2015.004.
3. Nadkarni AH, Nair SB, Italia KY, Warang P, Dalvi M, Ghosh K, Colah RB. Molecular Diversity of Hemoglobin H Disease in India. *Am J Clin Pathol* 2010; 133:491-4.
4. Shaji RV, Eunice SE, Baidya S, Srivastava A, Chandy M. Determination of the breakpoint and molecular diagnosis of a common a-thalassaemia-1 deletion in the Indian population. *Br J Haematol*. 2003; 123:942–7.
5. Desai SN, Colah RB. Alpha-thalassemia syndromes in India. Indian J Hum Genet. 1997; 3:1-9.
6. Mishra AK, Tiwari A. Alpha Thalassemia – A Rare but Perilous Blood Disorder. *Int. J. Med. Public health*.2012; 2:1-5.
7. Chui DHK, Fucharoen S, Chan V. Hemoglobin H disease: not necessarily a benign disorder. Blood 2003; 101(3):791-99.
8. Tahlan A, Khurana U, Palta A, Chauhan S. Hb H disease: An under diagnosed entity in Indian setup. *Indian J Pathol Microbiol*. 2009; 52(3): 451-2.
9. Balgir RS. The burden of haemoglobinopathies in India and the challenges ahead. Current Science. 2000; 79:1536-47.