Mild to Severe Anemia of Undiagnosed Etiology Diagnosed by Genetic Study

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Abstract

We report a case of a child with beta thalassemia major, whose mother is a carrier of beta thalassemia and father is having hereditary persistence of fetal hemoglobin. *Gene study revealed compound heterozygous for codon 8/9+G and IVS-1-5 G>C point mutation.* Another four cases of anemia not responding to iron *diagnosed to have alpha thalassemia carrier are also reported here.*

Introduction

Beta thalassemia affects child when both parents are carriers. Rarely Thalassemia Major or Intermedius is reported in children in whom one parent is a carrier of beta Thalassemia and the other parent has hereditary persistence of fetal haemoglobin due to new mutation. Alpha thalassemia carrier state is not very rare-single gene deletion is detected in 30% African Americans but often they are mistaken as iron deficiency anemia especially if genetic study is not conducted.

The Case

A female child of six months, of non-consanguineous marriage with no family history of anemia,presented with severe pallor, progressive abdominal distension. On examination child had respiratory distress with signs of congestive heart failure. Anthropometry were between (-2) to (-3) Z score. Her abdominal examination revealed soft palpable liver, 6 cm below costal margin with a span of 11 cm; and a firm spleen of 5cm. The laboratory investigation showed; hemoglobin (Hb): 4.2 gm/dl, total white blood cell count (WBC): 19,670/cumm, Platelet: 130,000/cumm, mean corpuscular volume (MCV): 65.6fl, mean corpuscular hemoglobin (MCH): 17pg/cell and mean corpuscular hemoglobin concentration (MCHC): 22.3 g/dl. Peripheral smear showed severe anisopoikilocytosis, microcytes, hypochromia, tear drop cells, fragmented cells. The NRBC were seen in ratio of 460/100 WBC. The iron work up was within normal limits. The liver function test (LFT) except indirect bilirubin, creatinine, and viral markers (Hepatitis B, C and HIV) were normal.

Echocardiography showed dilated left ventricle with global hypokinesia. Hemoglobin electrophoresis using high performance liquid chromatography (HPLC) of the patient and parents were sent. The peripheral smear and HPLC of Mother showed Hb: 9g/dl, HPLC

showed HbA2: 6.4%. The HPLC of father showed Hb: 12.8g/dl, HbA: 73.6%, HbA2: 2.4% and HbF: 14.8%. Both parents had normal bilirubin. The HPLC of the baby showed HbA: 5.1%, HbA2: 2.2% and HbF: 94.7%. The mother was a carrier of beta thalassemia but the father had hereditary persistence of fetal hemoglobin (HPFH) yet still their child was having features of thalassemia major.

Causes of elevated HbF excluding HPFH are Fanconi anemia, Diamond Blackfan Syndrome, congenital leukemia, delta-beta thalassemia, but in all cases patient will be symptomatic. Sometimes due to presence of associated delta thalassemia HbA2 may not rise (compound heterozygous) but in that case also the patient should have anemia of mild to moderate degree. Gene report showed compound heterozygous for codon 8/9+G mutation and IVS-1-5 G>C point mutation. Mutation analysis of parents could not be done due to financial constrain.

We took our case as Thalassemia major rather than intermedius as the child presented at the age six months and she needed repeated transfusion in the eight month follow up. Four other cases of undiagnosed anemia were also diagnosed by genetic study. Ages of the patients were between six months to four years, two were males. One was Muslim and others were Hindu. They had hemoglobin between 8g/dl to 10.1g/dl. MCV ranging from 61fl to 68fl. Ferritin 42 to101 microgram/l; HBA2 levels were 2.3% to 3%. Parents of all of them had normal hemoglobin electrophoresis (Table2). They were referred to us for anemia not responding to iron therapy. Reticulocyte count, bone marrow study, WBC count, C- reactive protein (CRP), creatinine values were normal. Lead levels in blood were normal.Gene analysis revealed- three of them having -a3.7/-a4.2 compound heterozygous mutation, one had -a3.7 heterozygous mutation. Genetic analysis of parents was possible in only one case. Mother had compound heterozygous –a3.7/-a4.2 and father had –a4.2 mutation.

Discussion

Beta thalassemia syndromes are heterogeneous group of hereditary disorder characterized by reduced or absent beta globin chain synthesis that affect the biosynthetic imbalance between alpha and non- alpha globin chain leading to ineffective erythropoiesis and anemia[1]. Till date more than 810 entries of beta thalassemia alleles have been identified[2]. Most of them are nucleotide substitution and frame shift or small mutation. When two mutations present as homozygous or compound heterozygous state major or intermedius form of the disease manifest. Common beta globin mutations are IVS 1-5 (G>C), codon 41/42(-TTCT), IVS 1-1(G>C), codon 17(A>T), IVS-II-654, codon 71/72(A+), G 8/9. (2), (3) IVS 1-5 (G>C) mutation is the commonest one in India.

Table 1: The blood parameters of the first patient

Parameters	Patient	Mother	Father
Age/Sex(M/F)	6months/F	22yr/F	24yr/M
Hemoglobin(g/dl)	4.2	9	12.8
PCV(%)	15	32	45
MCV(fl)	65.6	67.5	78.9
MCH(pg)	17	18	22.3
MCHC(g/dl)	22.3	27.6	28.3
RDW-cv(%)	25.5	14.2	17.4
HbA2(%)	2.2	6.4	2.4
HbF(%)	94.7	1.2	14.8

Table 2: Blood parameters of the four other patients

	Patient I	Patient II	Patient III	Patient IV
Age(yr)	0.5	1	3	4
MCV(fl)	61	64.6	67	68
Ferritin	42	101	70	87.6
HbA2 (%)	3	2.9	2.6	2.3
Alpha mutation Study	-a3.7	-a3.7/-a4.2	-a3.7/-a4.2	-a3.7/-a4.2
Parental Study	NA	F –a4.2;M-a3.7/-a4.2	NA	NA
Hemoglobin g/dl	10	10.1	9.8	9.2

Beta thalassemia present with a spectrum of clinical features depending on the beta gene mutations and coinheritance with other hemoglobinopathies. Heterozygous HPFH have an HbF level between10-35% and have benign course[4]. When these people marry and reproduce with another bearer of the beta globin mutation, the expression in offspring carrying a compound heterozygous genotype vary widely.In alpha thalassemia large deletions are common in contrast to beta thalassemia. Alpha thalassemia mutation may be non-deletional or deletional. Common deletions are –a3.7.-a4.2, SEA, THAI, if only one gene is deleted silent carrier state is diagnosed (-a/aa), if two genes are deleted (-a/-a trans) or (--/aacis) mild microcytic anemia will be detected. Three (--/-a HBH) or four (--/-- Bart HB) deletions are symptomatic with moderate to severe and even fatal anemia.

In our first case where the child presented with features of beta thalassemia major, the peculiarity was that, only the mother was the carrier of beta thalassemia and father was having hereditary persistence of fetal hemoglobin. The child had new mutation to behave like compound heterozygous form i.e. thalassemia major disease. Microcytosis can occur in iron deficiency, beta thalassemia (major, intermedius, carrier), sideroblastic anemia, lead toxicity, alpha thalassemia (carrier and disease).In three other cases two genes were deleted (-a3.7/-a4.2), this form is common in Africa and the forth case had 3.7 deletion. One amongst the four case whose parental genetic analysis was possible, the mother had two deletions and the father had one deletion. Usually in single gene defect, anemia is usually absent but in our case anemia was detected and no other cause of anemia was found. So without genetic analysis work up of anemia is not complete.

Conclusion

Beta Thalassemia major or intermedius can occur even if only one parent is a carrier due to new mutation. In case of unknown cause of microcytic anemia gene study for alpha mutation must be included.

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