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CASE REPORT ON RARE CASE PRESENTATION OF HAEMOGLOBIN E-BETA THALASSEMIC DISEASE IN HYDERABAD, INDIA.

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Abstract:
A 10 month old presented with the symptoms of jaundice and fever since 1 week. Routine investigations reveleaed hemolytic anemia and CE-HPLC & Whole exome Sequencing confirmed the diagnosis of compound heterozygous condition of Haemoglobin E-Beta thalassemic disease.
Reference words: HbE, Beta-Thalassemia, whole exome Sequencing, South India,

Introduction:
Globalisation- The solution and problem of the modern health care community. Immigration has helped solve many health problems while simultaneously bringing the rare diseases in to local communities.
This report describes a case of compound heterozygous HbE-Beta thalassemia.
HbE is the most common type of hemoglobinopathy in the world with higher prevalence in south east asia such as Thailand, Myanmar, Bangladesh, Srilanka . In India, it is more prevalent in north eastern states and relatively rare in south india. The disease presentation of HbE ranges from one to mild grade haemolytic anemia.
Beta Thalassemia is one of the commonest monogenic disorders in the world with many mutations known to cause this disease with varied severity. The causal genetic mutations vary with country, state, region, religion and culture.
Through this article I would like to emphasize the importance of increased awareness and attention in routine test analysis and follow up with genetic screening.

Case presentation:
A 10month old infant was brought to the Niloufer Hospital, Hyderabad with the complaints of yellow discolouration of eyes and fever as observed by parents for 7 days. the yellow

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discolouration was insidious in onset.it was gradual and slowly progressive. There was no discolouration of skin. There was no dark coloured urine but occasional clay-coloured stools. He was exclusively breast fed up to 6months and continued with complimentary feeds. The fever was intermittent and subsided with medications. The infant had no h/o reinfections, bleeding manifestations. No h/o blood transfusions or neonatal jaundice. He had no delayed milestones. There was no consanguinity in the parents' marriage. The mother suffered with mild anemia during pregnancy but did not require any blood transfusions or hospital treatment. Their family history enquiry was unsatisfactory.

The parents were revealed to be Rohingya muslim immigrants from Burma/Myanmar and have settled in Hyderabad for its large muslim community.

On general examination the patient was conscious and active. Mild icterus with mild pallor was observed. No clubbing, cyanosis, lymphadenopathy or pedal edema. He was afebrile during examination.

Systemic observation was normal with no frontal bossing or hepatosplenomegaly. Other system examinations were also normal.

Investigations:

As part of routine screening, Complete hemogram, Liver function test, Renal function testing and hormone, Ferritin & vitamin analysis was done along with beta thalassemia and sickle haemoglobin testing by Biorad D-10 variant HPLC.

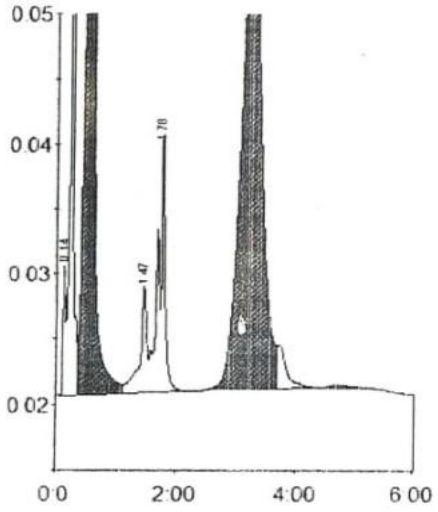
Total bilirubin: 4.45, Conj. Bilirubin: 0.85, Unconjugated Bilirubin: 3.85, ALP: 348.7, Vit.B12: 1852, Ferritin: 39.8. rest of the routine biochemistry was within normal limits.

Hb:7.3, RBC: 4.5M, MCV:54, MCH:16, MCHC:30, RDW:31%, WBC:22,300, Platelets:5.7L

Peripheral picture analysis revealed MicroCytic Hypochromic RBC, Target cells, Fragile RBC, Polychromatic RBC.

HPLC:

	Retention time	Area %
Unknown	0.14 min	1.1 %
HbA1a	0.25 min	9.0 %
Hb-F	0.55 min	36.3 %
P3	1.47 min	3.2 %
Hb-A0	1.78 min	7.6 %
HB-A2	3.23 min	49.3 %



Peak table - ID: 110

Peak	R.time	Height	Area	Area %
Unknown	0.14	10511	21665	1.1
A1a	0.25	43583	171720	9.0
F	0.55	77349	658756	36.3 *
P3	1.47	8134	62146	3.2
A0	1.78	19821	145041	7.6
A2	3.23	36689	857131	49.3 *
Total Area:		1916457		

VitaminB12: 1852pg/ml, Ferritin:39.8ng/ml

A provisional Diagnosis of haemolytic anaemia was made based on CBP, Peripheral blood picture. But elevated HbF and HbA2 on HPLC is not seen with any kind of haemolytic anaemia. Hence the probability of other haemoglobinopathy was suspected and so further testing of both parents was done.

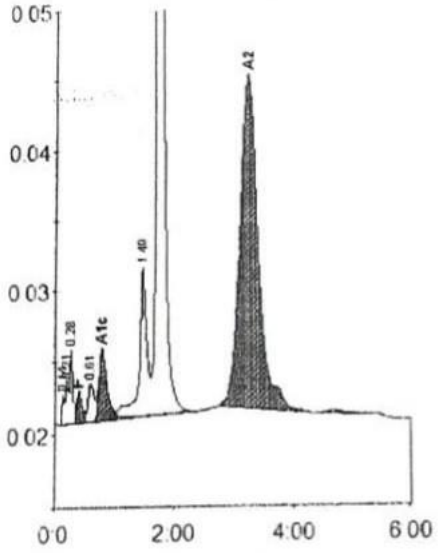
Father(27y):

Hb:13.9gm%, RBC: 6.4M, MCV:66, MCH:22,MCHC:32, RDW: 31%,WBC:18,000,Platelets:4.3L

Peripheral picture analysis revealed MicroCytic Hypochromic RBC, Nucleated RBC.

HPLC:

	Retention time	Area %
Unknown	0.14	0.3 %
HbA1a	0.21	1.1%
HbA1b	0.28	0.8 %
Hb-F	0.41	0.8 %
LA1c/CHb-1	0.61	1.0 %
HbA1c	0.80	4.7 %
P3	1.49	4.8 %
HbA0	1.74	63.4 %
HB-A2	3.28	28.9 %



Peak table - ID: 127B

Peak	R.time	Height	Area	Area %
Unknown	0.14	1992	4955	0.3
A1a	0.21	3071	11575	0.6
A1b	0.28	5107	15035	0.8
F	0.41	2118	14151	0.8
LA1c/CHb-1	0.61	2595	19115	1.0
A1c	0.80	4959	43876	4.7
P3	1.49	10398	91527	4.8
A0	1.74	269602	1205496	63.4
A2	3.28	23641	495637	28.9 *
Total Area:	1901367			

Sugge

VitaminB12: 827pg/ml, Ferritin:53.1ng/ml

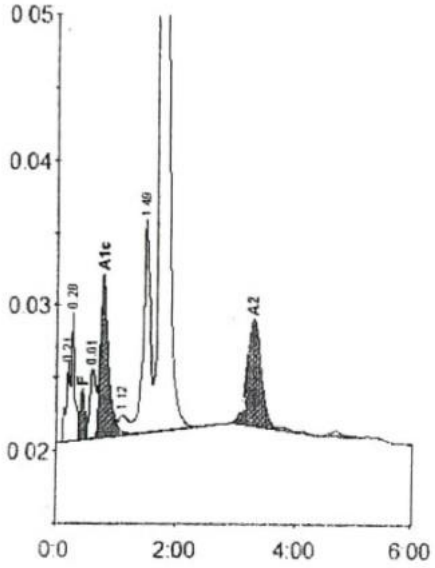
Mother(23y):

Hb:11.1gm%, RBC: 5.3M, MCV:70,MCH:21,MCHC:30,
RDW:14%,WBC:7,800,Platelets:2.8L

Peripheral picture analysis revealed MicroCytic Hypochromic RBC

HPLC:

	Retention time	Area %
A1a	0.21	1.1 %
Hb-A1b	0.28	1.1 %
Hb- F	0.44	0.8 %
LA1c/Chb-1	0.61	1.4 %
HbA1c	0.79	5.6 %
Unknown	1.12	0.6 %
P3	1.49	4.6 %
Hb A0	1.73	81.8 %
Hb A2	3.29	5.4 %



Peak table - ID: 127A

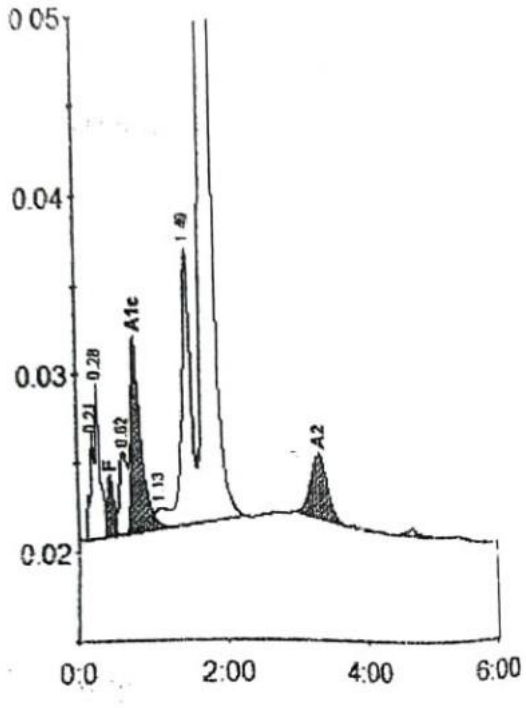
Peak	R.time	Height	Area	Area %
A1a	0.21	5480	28734	1.1
A1b	0.28	8831	26950	1.1
F	0.44	3424	19232	0.8
LA1c/CHb-1	0.61	4737	34266	1.4
A1c	0.79	11069	97209	5.6
Unknown	1.12	1299	14280	0.6
P3	1.49	14648	117236	4.6
A0	1.73	434591	2068800	81.8
A2	3.29	7373	121882	5.4
Total Area:	2528588			

VitaminB12: >2000pg/ml, Ferritin:95.1ng/ml

Elder Sister(3y):

CE-HPLC

	Retention Time	Area %
HBA1a	0.21	0.9 %
HbA1b	0.28	1.5 %
Hb F	0.44	1.0 %
LA1c/Chb-1	0.62	1.5 %
HbA1c	0.79	6.8 %
Unknown	1.13	0.5 %
P3	1.49	5.3 %
Hb A0	1.73	82.7 %
Hb A2	3.28	3.0 %



Peak table - ID: 139

Peak	R.time	Height	Area	Area %
A1a	0.21	6189	22541	0.9
A1b	0.28	8677	37533	1.5
F	0.44	3425	21146	1.0
LA1c/CHb-1	0.62	4643	37294	1.5
A1c	0.79	10847	98664	5.8
Unknown	1.13	1192	12404	0.5
P3	1.49	15620	129186	5.3
A0	1.73	421443	2007478	82.7
A2	3.28	3772	60390	3.0
Total Area:	2426636			

Testing in the elder sibling revealed normal blood picture, HPLC and normal routine biochemistry levels. She was asymptomatic to date.

Based on the presence of Microcytic, Hypochromic blood picture, and abnormal HPLC picture- elevated HbF and HBA2, a provisional Diagnosis of HbE in father and Beta Thalassemaia trait in mother was made.

After the analysis of parents blood pictures and CE-HPLC results, provisional diagnosis of HbE-**β** thalassemia was made in the patient.

Hb Electrophoresis was not done due to lack of equipment availability.

Samples for genetic testing, to confirm the provisional diagnosis was sent to Institute of Genetics and Hospital for Genetic Diseases-Hyderabad, for whole exome sequencing of haemoglobin gene on chromosome 11 in the patient and parents.

Exome Analysis of HBB gene in Patient and Parents:

Father: HBE : c.79G>A (p.Glu27Lys)

Mother: HBB : c.124_127delTTCT [codons 42/43 (–TTCT)] (p.Phe42LeufsTer19)
Patient: HBB:NM_000518.4: c.124_127delTTCT [codons 42/43 (–TTCT)] (p.Phe42LeufsTer19) and HBE: c.79G>A (p.Glu27Lys).

Differential Diagnosis:

The differential diagnosis of the current condition included HbD-Iran, HbD-Punjab, Beta thalassemia major, Sickle cell Anemia, Hereditary persistence of fetal hemoglobin (HPFH).
HbD- Iran have a retention time of 2.91 min on Biorad D-10 CE-HPLC and commonly seen in people of western asia and north west India [1] and hence excluded.
HbD Punjab shows unknown peak at 3.9min retention time and does not elute with HBA2.
Beta Thalassemia Major was excluded due to high A2 and only moderately raised HbF. HPFH was also excluded for the same reason.
Treatment:
The patient was given packed cells transfusion. He was referred to a hematologist who advised regular transfusions and observations upto 2 years and probable Bone marrow transplant after 2years.
Genetic councelling was given to the parents for future complications of the HbE disease and also for future pregnancies.

Discussion:

CE-HPLC:
There was unknown peak at 0.14 min in both patient(1.1%) and father(0.3%) of the patient. It was absent in both mother and sister. Its peak area was considerably higher in patient.
HbA1a one of the minor glycated haemoglobin products was found abnormal with higher retention time(0.25min) and increased peak area(9.0%) in the patient. It had a retention time of 0.21min in both father, mother and sibling.
HbF, fetal haemoglobin was higher in the patient and eluted at delayed time of 0.55 min where as the normal time of elution was 0.41 min.
Minor hb fractions like HbA1b and cHb/LA1c were completely absent in the patient along with HBA1c. this was contradictory to the report given by vani et al, who found higher concentrations of HbA1b in HbE-**β** thal patients.
The elevation of HbA2 in patient(49.3%) and father(28.9%) correlated with the previously reported levels of HbE-**β** thal and HbE.[2]
False Elevated HBA2 levels are seen with Vitamin B12 defeciency[3]. But the none of the patients analysed had any B12 defeciency
Our case is showing A2 peak with 48.9% and retention time of 3.28 minutes which suggests HBE trait or HbD Iran. But based on retention time, peripheral picture and ethinicity we made a provisional diagnosis of HbE Trait rather than HbD Iran. Genetics confirmed the diagnosis of HbE-**β** thalassemia.
HBB Gene:
Haemoglobin is made of 4-Alpha chains and 2-Beta chains. Any mutation in the genes coding for these chains result in decreased production and cause condition known as Thalassemia. Depending on the number alleles affected the disease severity varies. The

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disease caused by mutation in the beta globin genes is known as Beta Thalassemia. Beta globin gene in humans is located on the chromosome 11, short arm at location 15.4.

Many different kind of mutations are seen in beta globin gene. More than 350 disease causing mutations are recorded in beta globin gene. These mutations may be of any kind insertion/ deletion, in the exons and introns of the gene along with mutations in the promotor region. So far only mutations in cis acting transcription factors have found to be causing the beta thalassemia disease.

Beta thalassemia is one of the most common hemoglobinopathies of the world. It is also one of the most common monogenic diseases affecting the world population. The mutations causing beta thalassemia change with country, region and also culture and community.

The most common β globin gene mutations seen in India are -IVS 1-5 G → C, IVS 1 -1 G → T, Codon 41/42 (- TCTT), Codon 8/9 and the 619 bp deletion account for over 90% of the mutations in β-thalassemia patients[4]. In the combined states of Telangana and Andhra Pradesh of south india, IVS 1-5 (G-C) was the commonest mutation with 73.15% of thalassemics having it followed by FS codon 15 (TGG-TAG), which was seen in 13.05% of the cases. The frequency of other common mutations were deletions of codon 41/42 in 7.63%, codon 8/9 in 3.44%, codon 16 in 2.46% and 619bp (0.246%)[5].

In HBB:NM_0005 18.4: c.124_127delTTCT [codons 42/43 (–TTCT)] (p.Phe42LeufsTer19) mutation, there is deletion of four nucleotides and frame shift of the genes resulting replace ment of phenylalanine by leucine at 42 position and also other aminoacids. The amino acid chain termination also occurs early at 61 amino acid resulting in short abnormal dysfunctional beta globin chain. It is a common pathogenic genetic mutation of beta thalassemia.

Though c.41/42 is common mutation India and Myanmar(23%)[6]. It very less common in south india. Its incidence is less than <1% in the state of Telangana[8].

Hb.E: Mutation in c.79G>A (p.Glu27Lys) is a missense SNP mutation where Guanine is replaced by Adenosine. This causes a change in single aminoacid Glutamic acid to Lysine. This change in amino acid activates cryptic splicing site in pre-mRNA along with a reduction in normal splicing resulting in decreased beta globin chain synthesis. This is almost a similar picture as that of beta thalassemia. But in HbE disease,it seems, the cryptic splice site is not always activated.(Depending on the number of chromosomes affected the amount of abnormal haemoglobin and clinical picture varies.)

HbE disease is commonly seen in south east Asian countries like Myanmar, Thailand etc. In India, HbE is highly prevalent (7.0–50.0%) in the northeastern region and 1.0–2.0% in West Bengal[2]. Studies show lesser incidence of HbE in south India and Telangana. The incidence in south India varies with states (1-23%)[2].

These studies cannot be considered accurate regarding HbE as most patients with HbE disease- heterozygous or homozygous show very little signs and symptoms. And in a country with high prevalence of anemia, and abnormal haemoglobin can be easily dismissed as nutritional anemia rather a genetic condition. This seems to be the situation in the mother of the patient where despite many incidences of anemia, in routine testing and pregnancy, the cause was never seriously pursued.

HbE mutation when combined with beta thalassemia results in condition-HbE/β thal that closely resembles beta thalassemia major. It is one of the severe forms of both diseases.

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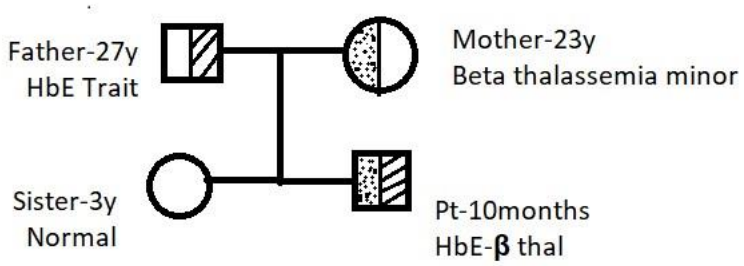
Pathophysiology of Hb E/β-thalassaemia is related to many factors including reduced β chain synthesis resulting in globin chain imbalance, ineffective erythropoiesis, apoptosis, oxidative damage and shortened red cell survival. In general, it appears that the recognized instability of haemoglobin E is a minor factor in the overall pathophysiology of Hb E/β-thalassaemia, except during intercurrent febrile illnesses during which such instability may result in accelerated haemolysis [7].

The patient is identified with compound heterozygosity of both beta thalassemia and haemoglobin E disease inherited from both parents. His clinical scenario and haematology picture is significant when compared to parents. As the production of fetal haemoglobin decreases during late infant period and there is no proper production of beta globin chains due to mutation in both alleles of HBB genes of both chromosomes, there is phenotypic expression of severe anemia as seen in the patient.



Learning Points:

Though the immigration is long occurring situation, the information regarding the diseases is not much shared among the physician community located in all areas.

HbE is one of the common mutations in India. Despite this, its awareness is very low in physicians and biochemists. Many of such cases are misdiagnosed as beta thalassemic variants because of its low clinical severity and its elution with hbA2 in HPLC analysis.

Analysis of HbE with the help of D-10 variant model are very low. As the retention time of hba2 changes with model, it is important to know the specific time for each variant in different HPLC models.

Thorough prenatal screening is very important to identify and rectify the anaemia in pregnant women a. This will also help in early identification and effective prevention of anemia in the mother and the child. CE-HPLC is an easy and cost-effective procedure for identification of the various haemoglobinopathies. We Also highlight the importance of exome sequencing for haemoglobinopathies.

References:

1. Bhat VS, Mandal AK, Mathew B. Co-inheritance of HbD (Iran)/beta Thalassemia IVS1-5 (G > C) trait in a Punjabi lady with diabetes. Indian J Clin Biochem [Internet]. 2012;27(2):202–6. Available from: http://dx.doi.org/10.1007/s12291-012-0201-3

2. Chandrashekar V, Soni M. Hemoglobin disorders in South India. ISRN Hematol [Internet]. 2011;2011:748939. Available from: http://dx.doi.org/10.5402/2011/748939

3. Warghade S, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65779 cases. J Lab Physicians [Internet]. 2018;10(1):73–9. Available from: http://dx.doi.org/10.4103/JLP.JLP_57_17

4. Panigrahi I, Marwaha RK. Mutational spectrum of thalassemias in India. Indian J Hum Genet [Internet]. 2007;13(1):36–7. Available from: http://dx.doi.org/10.4103/0971-6866.32034

5. Iyer, Gayatri & Kamireddy, Aruna & Kondaveeti, Saroja & Jain, Suman & Hasan, Qurratulain. (2019). Establishing Mutational Spectrum of Beta Thalassemia by Molecular Screening in a Low Resource Setting-Implications in Counseling and Prevention. Vol. 4,. 36-43.

6. Ne Win.Comparative molecular spectrum of beta-thalassaemia mutations in Myanmar with neighbouring countries. Myanmar medical journal. September 2019 - Vol: 61, No. 3.

7. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: a common & clinically diverse disorder. Indian J Med Res. 2011;134:522–31.

8. Munshi A, Anandraj MPJS, Joseph J, Shafi G, Anila AN, Jyothy A. Inherited hemoglobin disorders in Andhra Pradesh, India: a population study. Clin Chim Acta [Internet]. 2009;400(1–2):117–9. Available from: http://dx.doi.org/10.1016/j.cca.2008.10.025