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A CASE OF PNEUMONIA WITH RARE Hb E VARIANTS

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ABSTRACT
We describe a case of a boy of 2 years six months with pneumonia and HbE/B^0thal. The patient presented with high grade fever associated with chills and rigors. As the patient was previously labeled as thalassemia minor and Hemoglobin on admission was 5.6 gm/dl sample was sent for Agarose gel alkaline (ph-8.8)Electrophoresis and High performance liquid chromatography. Both unexpectedly revealed hemoglobin at HbA2 position/window. As neither electrophoresis nor BioRad variant Hb HPLC can differentiate between HbA2 and HbE, HPLC with modified mobile or stationary phase and protocols are needed for complete diagnosis.

KEY WORDS: Thalassemia,Hb E,Hb A2

INTRODUCTION:
$2^{1/2}$years male presented with chief complains of high grade fever since last 2 days, associated with chills and rigors. Patient also has complained of cough. Patient was a known case of thalassemia minor and also had past history of 2 times blood transfusion. Clinically Patient is pallor and icteric. Patient also shows features of thalassemia like frontal bossing, hemolytic face *etc*. Complete Blood Count Show elevation of total WBC Count. Agarose gel alkaline (ph-8.8) Electrophoresis and High performance liquid chromatography was done. It was suggestive of elevation of HBA2 level (51.40%) (Normal level 1.5-3.2%) and HBF level (42.50%) (Normal level 0-2%).
Chest x-rays shows areas of opacity (seen as white) which represent consolidation.

MATERIAL & METHOD
We report a case of Pneumonia with abnormal Hemoglobin. Patient's blood and urine was analyzed in Hematology, Microbiology and Biochemistry laboratories of new civil Hospital, Surat.

RESULTS

Examination	Result
Hb	5.6 gm%
Total RBC	3.09 mill/cmm
PCV	21%
MCV	68%
MCH	18.12 pg
MCHC	26.67%
RDW	37%
Total WBC	17,000 /cmm
Reticulocyte	12%
Hypochromia ++, Anisocytosis +++, Microcytes ++, Macrocyte+, Poikilocytosis +++, Basophilic stippling, Fragmented RBCs	
Agarose Gel Electrophoresis	Dark band at HbA2 and HbF place(Figure-1)
HPLC	HbA2: 51.40%, HbF 42.50%(Figure-2)

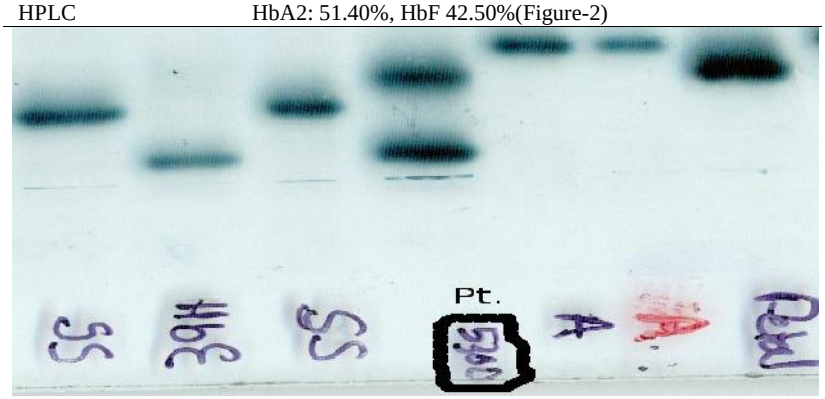

FIGURE1: Agarose Gel Alkaline Electrophoresis(pH 8.8) of patient

FIGURE2: Agarose Gel Alkaline Electrophoresis (pH 8.8) of mother and father of the patient

Peak Name	Calibrated Area%	Area%	Retention Time (min.)	Peak Area
F	42.5*	---	1.14	421521
P3	---	2.2	1.83	23874
Unknown	---	2.2	2.28	24334
Ao	---	4.3	2.51	46884
A2	51.4*	---	3.72	569575

Total Area : 1,086,188

F Concentration = 42.5* %
A2 Concentration = 51.4* %

Analysis comments:

*Values outside of expected ranges

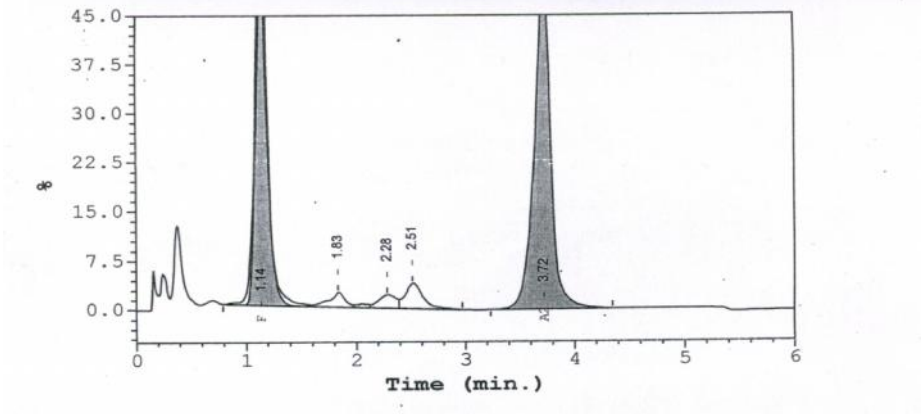

FIGURE 3: HPLC of the patient's blood elevation of Hb F and HbE (HbA2 window)

Dark band at HbA2 in alkaline Hb Electrophoresis and Peak at HbA2 window together indicate presence of HbE in the patient as HbA2 is never so high in any condition and only HbE co-elutes with HbA2. Total absences of HbA in this patient together with elevated HbF indicate either HbE/E or HbE/B^0thal. Hb Electrophoresis of the mother of this patient show HbA2 and HbA bands indicating HbE carrier state of mother. Hb Electrophoresis of the father of this patient show HbA band, but quantitative HbA2 result was not available.

DISCUSSION

Hemoglobin E. Hemoglobin E is a structural variant of the hemoglobin beta chain and is the most common such variant among Southeast Asians. The gene frequency varies considerably between regions but is generally 1/10 to 1/20 and may reach as high as 1/2 in some regions near the intersecting borders of Cambodia, Thailand, and Laos. It is also prevalent in parts of the Indian subcontinent. In contrast, hemoglobin E is much less common in the Vietnamese population and is almost absent among the Chinese and Japanese populations. Hemoglobin E comprises 2 normal alpha chains and 2 variant beta chains in which lysine has replaced glutamic acid at position 26. ($\alpha 2\beta 2^{26}$ Glu-->Lys) (4) Hb E may present as Homozygous state(EE) (5)or Heterozygous state(EA)(5) or compound heterozygous state with thalassemia(E/B^+thal,E/B^0 thal)(6),sickle cell disease(ES).

Biochemical basis of HbE

β chain of Hb E is synthesized at slow rate because mutation also creates alternate splicing site on hn-RNA which lead to decrease synthesis of functional mRNA and decreased translation of β globin. HbE may therefore be regarded as a β+thalassemic hemoglobinopathy.

Variation of HbF level in Hb E/*B*-thalassemia (7)

Hb F level predictor of morbidity. Xmn I(+) polymorphism in promoter region of gene of gamma globin may be responsible for increase Hb F level.(3)Severity of disease is related to type of *B*-thalassemia mutation Co-inheritance of α thalassemia decrease globin chain imbalance, improve anemia.

Major problem in complete diagnosis of such cases is lack of commercially available HPLC protocols and equipments which can further resolve hemoglobin variants beyond that provided by Biorad HPLC equipments.(8)

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