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Letter to Editor

Pathology Section

Significance of Early Onset Peaks on High Performance Liquid Chromatography

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Keywords: Electrophoresis, Hemoglobinopathies, Target cells

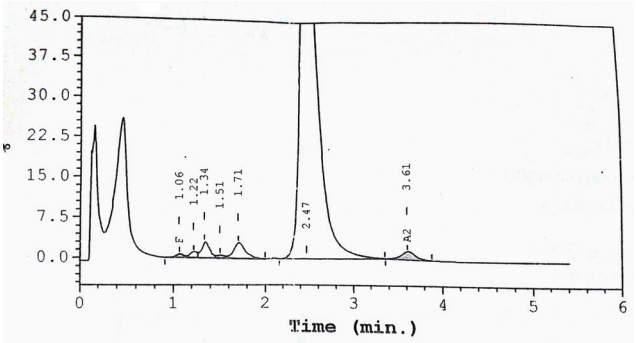
Sir,

High Performance Liquid Chromatography is a rapid and accurate mean for early diagnosis of various hemoglobinopathies. However, availability and applicability of HPLC in Indian scenario is very fragmented and non uniform.

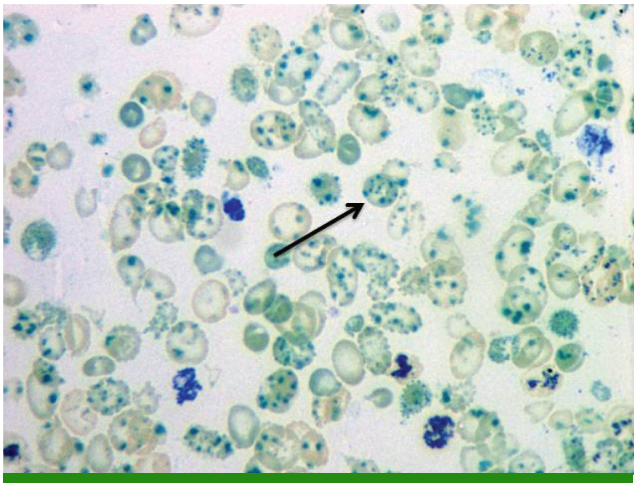
Alpha - thalassemias are group of hemoglobinopathies with defective α chain synthesis. Diagnosis of α-thalassaemias trait can be difficult as there are no abnormalities detectable by haemoglobin electrophoresis or high performance liquid chromatography (HPLC). We report here two cases of HbH diagnosed with the help of HPLC and supravital staining. Both the cases were children and presented with pallor and hepatosplenomegaly. Their haematological indices are shown in [Table/Fig-1]. On peripheral smear examination, both of them showed microcytic hypochromic red cells with marked anisopoikilocytosis and many polychromatophils. Few target cells and occasional nucleated red cells were also present. HPLC (Biorad variant II) in both these cases showed sharp significant peaks before the start of integration and low HbA2 [Table/Fig-2]. Reticulocyte preparation with 1% new methylene blue showed reticulocytosis along with typical golf ball inclusions [Table/Fig-3]. These patients were

	Case 1	Case 2
Age (in years)	2.5	3
Clinical Features	Pallor and hepatosplenomegaly	Pallor and hepatosplenomegaly
Hb (g/dl)	3.9	7.0
MCV (fl)	61.4	62.2
MCH (pg)	15.1	16.1
MCHC (g/dl)	24.5	25.8
RDW (%)	33.5	41.6
Reticulocyte Count (%)	11.8	6.3
HbA2	2.1	1.6
HbF	0.8	0.4

[Table/Fig-1]: Showing clinical details and haematological indices of two children.



[Table/Fig-2]: HPLC showing sharp significant peaks before the start of integration.



[Table/Fig-3]: Reticulocyte preparation with 1% new methylene blue showing reticulocytosis along with typical golf ball inclusions.

provisionally diagnosed as HbH disease and family studies, Hb electrophoresis and globin chain analyses were advised. Family study was available in only one case (case no 2) as the other child was an orphan. Mother of the child showed normal chromatogram however father of the child showed decreased HbA2 on HPLC along with golf ball inclusions in approximately 10% of red cells.

HbH disease is a common entity in Southeast Asia with the

frequency varying from 4% to 30% in varying studies [1,2] however, is usually under-reported in Indian subcontinent [3]. HbH dise`ase is caused by the presence of only one functional α gene which occurs due to compound heterozygous state for α 0/ α + thal (--/-α /--/ α T α). Decreased α chains and relative excess of β chains leads to formation of tetramers of βglobin chains (HbH). This HbH is unstable and precipitates in red cells causing hemolysis. Also high oxygen affinity leads to hypoxia disproportionate to Hb level [4].

Hence, in under resourced laboratory, where gene analysis is not possible, reticulocyte preparation showing typical inclusions and HPLC can help in early detection of HbH disease.

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