Original Research Article

Study of Prevalence of Thalassemia and its variants using HPLC – A Hospital based Retrospective Study

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Abstract

Background: Structural defect in haemoglobin are the most common inherited abnormalities of hemoglobin synthesis. **Objective:** Early and accurate diagnosis of hemoglobinopathies. Diagnosis of these disorders through HPLC is most convient investigation for diagnosis of hemoglobinapathies. **Results and conclusion:** Abnormal hemoglobin fractions on HPLC were seen in 338 cases of total 730 samples examined. Out of all the cases, β Thalassemia Minor was the predominant abnormality. 75 cases (10.27%) were β Thalassemia Major and 17 cases (2.325%) were β Thalassemia Intermedia. 2 cases with diagnosis of Sickle β thalassemia were reported. In our study, one case of δβ thalassemia and 4 cases of δβ thalassemia trait were also reported. HPLC is easy and convient method to rule out hemoglobinopathies. The trend of labelling the diagnosis of hemoglobinopathy with HPLC instead of Hb Electrophoresis is rapidly rising. Thus, HPLC is a better tool to rule out hemoglobinopathy and improve the life standards of general population.

Keywords:β thalassemia, Hemoglobinopathies, High Performance Liquid Chromatography

Introduction

Hemoglobinopathies are the result of structural defect in hemoglobin synthesis. Quantitative defect in globin chain synthesis of hemoglobin results in Thalassemia. These are the commonest single gene disorders globally with an autosomal recessive inheritance and it is estimated that around 300,000 to 400,000 babies with a severe hemoglobin disorder are born each year [1]. According to world health organization (WHO), 5% of the world population is a carrier for Hemoglobin disorders [2]. India has a wide diversity of popupation. Different parts of the country has difference in prevalence of β-Thalassemia: 6.5% in Punjab, 8.4% in Tamilnadu, 4.3% in south India, and 3.5% in Bengal. β- Thalasemia has a high prevalence in some communities, such as Sindhi, Luvana and Rajputs [3]. The expected annual births of β - Thalassemia major babies was also calculated for each district in gujarat state. The rate of homozygosity per 1000 births annually was 0.39 in Gujarat [4]. Diagnosis of Thalassemia was made considering clinical profile, family history, geographic location of patient, complete blood counts (CBC), peripheral smear examination, red cell indices, HbA2 level, HbF estimation, Hb electrophoresis and HPLC estimation. An increase levels of HbA2 with an average value of about 5%, along with blood picture suggestive of microcytic hypochromic red blood cells, is characteristic of β-thalassemia trait [5]. In β- thalassemia major, levels of HbA2 are very high along with increased levels of HbF ranging from 10 to 90% in addition to peripheral smear showing microcytic hypochromic blood picture [5].

Materials and Method

A Retrospective study was carried out over a period of 1 year in HPLC Section, Pathology Department of M P Shah Government Medical and Hospital, Jamnagar– A tertiary care centre. The total samples collected during this period as per our inclusion criteria were 730. EDTA blood samples were collected from the patients with detailed personal history, family history and geographic location of the patient. Samples received were run in ADAMS HA – 8180T Hemoglobinopathy/HbA1c Analyzer of ARKRAY company. The machine works on the principle of reversed – phase cation exchange chromatography. The guidelines to assure acceptable reults :

1) The instrument has passed calibration.
2) The instrument can give acceptable QC result.
3) Total are of A0 should be 20,000 – 60,000.
4) Base line should be clear.
5) The reportable ranges: HbA1c: 3% to 20%, HbF: 0 to 100%.

Eluents A, B and C were used in the machine.
Eluent A elutes the area before A0 and stabilizes the column
Eluent B elutes A0 and A2
Eluent C elutes variant Hb (HbS, HbD, other)

The graph is generated (Fig 1) and depending on the time of elution of Hb peak, graph is interpreted according to Elution Times T model given as reference literature with the machine (Table 1).

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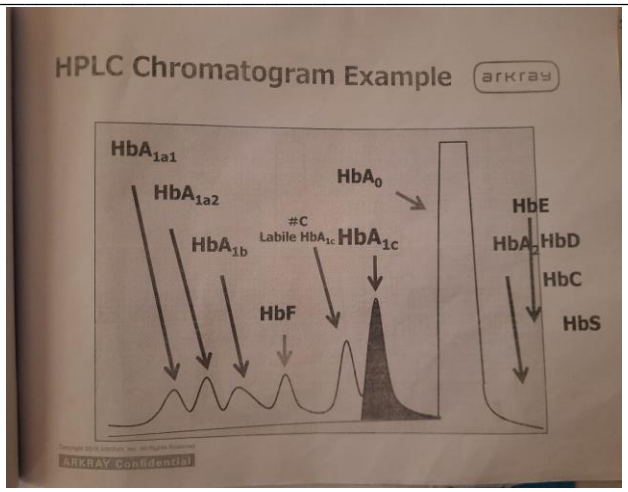


Fig 1: HPLC Chromatogram showing elution of different haemoglobin.

Fig 2: Elution Times T model

Fraction	Sec	Remarks
HbBarts	5	1st peak, usually HbF will aslo be high
HbH	5	1st peak...usually seen along-with HbCS
HbF	13	
L-A1c	18	
S-A1c	21 - 31 (26)	
A0	73-77	
HbA2	100-108	Same time as A2...hence A2 result not given if HbE is present. If peak is (>10%) then it is HbE
HbE	100-110	Appears immediately after A2 and sometimes A2 result not outputted
HbD	140	Same time as HbS but HbTak peak is bi-modal, main peak seen before sub-peak
HbTak	140-160	
HbS	160-163	
HbCS - Constant Spring)	182	After A2 peak. Indicator of Alpha thallasemia
HbC	180	Completely separated
HbLepore	90	Occurs between A0 and A2...since it overlaps with A2, the A2 value is not outputted
HbJ / HbJ Bangkok	59-60	Usually bimodal peak of A0 - 1st peak is HbJ

Observation and Result

A total of 730 samples were examined for HPLC. Out of all the samples received for suspected cases of hemoglobinopathy, 392 cases (53.86%) were having normal levels of haemoglobin and 338 cases (46.30%). 190 cases (26.02%) were β Thalassemia Minor, 75 cases (10.27%) were β Thalassemia Major and 17 cases (2.325) were β Thalassemia Intermedia. (Table 2). 2 cases with the diagnosis of Sickle β Thalassemia were reported . 1 case of δβ thalassemia and 4 cases of δβ thalassemia trait were reported as rare findings.

Table 1: Percentage distribution of Hemoglobinopathies

Sr.No	Patterns of Hemoglobinopathies observed	No of cases	Percentage(%)
1	Adult Hb & Fetal Hb Within Normal range	392	53.86
2	Adult Hb within normal range, HbF and HbA2 slightly raised	1	0.13
3	Hb F is high in K/C/O thalassemia major	1	0.13
4	HbF high for age/δβ thalassemia trait/HPFH	6	0.82
5	HPFH trait/β thalassemia intermedia	2	0.27
6	Homozygous δβ thalassemia/thalasseima intermedia/HPFH	4	0.54
7	HbF high for age/δβ thalassemia trait/HPFH	6	0.82
8	Sickle cell trait/β thalassemia trait	5	0.64
9	Sickle β thalassemia	2	0.27
10	β thalassemia carrier	1	0.13
11	β thalassemia intermedia	17	2.32
12	β thalassemia major with Hb Lepore	1	0.13
13	β thalassemia major/ δβ thalassemia/HPFH	1	0.13

14	β thalassemia major	75	10.27
15	β thalassemia minor	190	26.02
16	β thalassemia syndrome	1	0.13
17	β thalassemia minor with high HbA1c	1	0.13
18	β thalassemia syndrome / HbH variant	1	0.13
19	β thalassemia trait	15	2.05
20	δβ thalassemia/HPFH	3	0.41
21	δβ thalassemia	1	0.13
22	δβ thalassemia trait	4	0.54
	Total	730	100

Diagnosis of β thalassemia major was made with HbA2 levels 4.4% and HbF levels of 41.7%

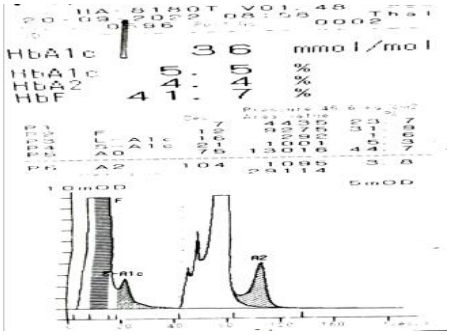
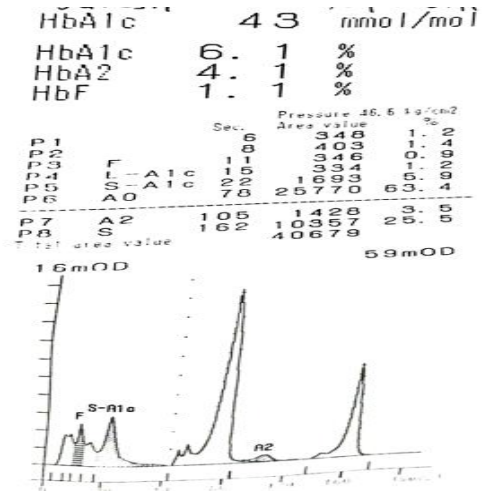
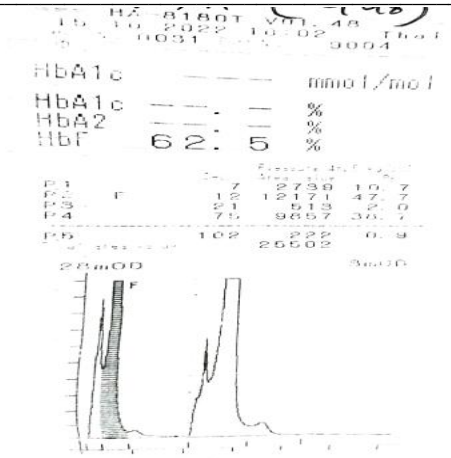


Fig 3: HPLC chromatogram showing β thalassemia major.



HbS levels 25.5%, HbF 1.1% and HbA2 4.1% were seen on HPLC chromatogram. Diagnosis was of Sickle β Thalassemia

Fig 4: HPLC chromatogram showing Sickle β thalassemia.



HbF was 62.5%. Depending on clinical picture, hemogram findings and HPLC graph diagnosis of δβ thalassemia was made.

Fig 5: HPLC chromatogram showing δβ thalassemia

Table 2: Gender wise distribution of various hemoglobinopathies

	Male	Female	Total cases
Adult Hb & Fetal Hb Within Normal range	192	200	392
Adult Hb within normal range,HbF and HbA2 slightly raised	0	1	1
Hb F is high in K/C/O thalassemia major	1	0	1
HbF high for age/δβ thalassemia trait/HPFH	2	4	6
HPFH trait/β thalassemia intermedia	2	0	2
Homozygous δβ thalassemia/thalasseima intermedia/HPFH	1	3	4
HbF high for age/δβ thalassemia trait/HPFH	2	4	6
Sickle cell trait/β thalassemia trait	2	3	5
Sickle β thalassemia	1	1	2
β thalassemia carrier	0	1	1
β thalassemia intermedia	8	9	17
β thalassemia major with hb Lepore	1	0	1
β thalassemia major/ δβ thalassemia/HPFH	1	0	1
β thalassemia major	46	29	75
β thalassemia minor	96	94	190
β thalassemia syndrome	0	1	1
β thalassemia minor with high HbA1c	0	1	1
β thalassemia syndrome / HbH variant	1	0	1
β thalassemia trait	8	7	15
δβ thalassemia/HPFH	2	1	3
δβ thalassemia	1	0	1
δβ thalassemia trait	4	0	4
Total	371	359	730

Table 3:Hb fractions in various hemoglobinopathies

Diagnosis	HbA0	HbA2	HbF
Adult Hb & Fetal Hb Within Normal range	93	3.07	0.839
β thalassemia intermedia	62.5	4.34	33.94
β thalassemia major	48.61	34.73	17.38
β thalassemia minor	90.03	5.38	1.35

β thalassemia trait	89.74	4.71	1.66
δβ thalassemia	72.8	3.5	23.7
δβ thalassemia trait	86.5	2.8	10.7

An increase levels of HbA2 with an average value of about 5%, along with blood picture suggestive of microcytic hypochromic red blood cells, is characteristic of β-thalassemia trait [5]. In β- thalassemia major, levels of HbA2 are very high along with increased levels of HbF ranging from 10 to 90% in addition to peripheral smear showing microcytic hypochromic blood picture [5]. In our study, the average value of HbA2 for β Thalassemia major was 34.73 and HbF was 17.38 whereas for β Thalassemia Minor and β Thalassemia Intermedia the value of HbA2 were 5.38 and 4.34 respectively and of HbF were 1.35 and 33.94 respectively (Table 3). δβ thalassemia had normal value of HbA2 but HbF was high. Hence depending on clinical findings and HPLC interpretation diagnosis of δβ thalassemia was made. In case of β thalassemia trait value of HbA2 was mildly increased.

Discussion

HPLC is emerging as one of the best methods for screening and detection of various hemoglobinopathies with rapid, reproducible and precise results [6]. In our Study, all the patients suspected for hemoglobinopathy were included. Total of 22 different diagnosis of haemoglobin variants were made from 730 samples received for the HPLC. Out of these, largest subgroup was of β thalassemia minor which was diagnosed in 190 cases (26.02%). The characteristic findings in patient of β thalassemia minor were absent or mild anemia, peripheral blood smear showing microcytosis, target cells and basophilic stipplings. The HPLC value of HbA2 was >3.5% and HbF < 5%. The CBC & HPLC parameters of the present study are in good correlation with the research conducted by Tejinder Singh,[7] Riou J,[8] & AllaJoutovsky.[9]

The highest frequency of beta thalasemia trait is reported in Gujarat (10-15%), followed by Sindh (10%), Punjab (6.5%), Tamil Nadu (8.4%) and Maharashtra [10]. Chopra et al. revealed that out of 1032 participant, 258 (25%) cases had abnormal haemoglobin [11]. However, Patel J et al. reported the prevalence of hemoglobinopathies in Gujarat, mentioning that out of 428 subjects, 153(35.7%) had Hemoglobinopathies [10] while there another study in year 2011 found higher prevalence up to 38.97%. [12]. The present study (Table 3) revealed higher prevalence of hemoglobinopathies in males 179/371 (48%) as compared to females 159/379 (41%).75 cases (10.27%) had diagnosis of β thalassemia major whereas 17 cases (2.32%) had diagnosis of β thalassemia intermedia. Incidental findings of 2 cases with diagnosis of Sickle β thalassemia were reported. In our study, one case of δβ thalassemia and 4 cases of δβ thalassemia trait were reported. The persistence of high levels of HbF in adults is seen in conditions such as δβ thalassemia and hereditary persistence of HbF[13],[14]. Findings of mild anemia with hemolytic features like thalassemia intermedia favor the diagnosis of homozygous δβ-thalassemia whereas patients of homozygous HPFH are asymptomatic with normal hematology profile [15],[16].

Conclusion

HPLC is easy and convient method to rule out hemoglobinopathies. The trend of labelling the diagnosis of hemoglobinopathy with HPLC instead of Hb Electrophoresis is rapidly rising. Thus, HPLC is a better tool to rule out hemoglobinopathy and improve the life standards of general population. Early detection of traits will prevent occurrence of thalassemia major in offspring.

Detection of other variants becomes important due to complex interactions in cases with double heterozygous and homozygous states, which may lead to severe hematological abnormalities.

Conflict of Interest: Nil Source of support: Nil

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