Spectrum of Hemoglobinopathies diagnosed by High Performance Liquid Chromatography: A cross sectional study

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Abstract
Background: **Inherited abnormalities of hemoglobin synthesis include a varieties of disorders. Identification of these disorders is immensely important epidemiologically and aid in prevention of more serious hemoglobin disorders.**

Aims: **High performance liquid chromatography (HPLC) forms an important tool for accurate diagnosis of various hemoglobin disorders. Early detection yields in better prognosis. About 150 cases have been studied for various hemoglobin disorders found in our region.**

Material and Methods: **The study was conducted in a retrospective manner and various findings like hemoglobin's and patients symptoms were noted. HPLC helps in better diagnosis.**

Results and Conclusion:
Abnormal hemoglobin fractions on HPLC were seen in 31 of the 150 cases studied. Of this, the beta thalassemia trait was the predominant abnormality with a total of 14 cases (9.33%). There were 1(0.66%) cases of beta thalassemia major. Sickle cell trait, sickle cell disease with one case of alpha chain variant were found. Detection of this abnormal hemoglobin, particularly keeping in mind a high prevalence of Hb A2, will help in prevention of more serious hemoglobinopathies including beta thalassemia major. Premarriage counselling and use of HPLC helps in early detection and management of various hemoglobin disorders.

Key words: **HPLC, hemoglobinopathies, hemoglobin variants, thalassemia**

I.Introduction:

Hemoglobinopathies are disorders affecting the structure, function, or production of hemoglobins. Hemoglobinopathies are one of the major public health problems in the state of Maharashtra, India. Hemoglobinopathies can be either quantitative or qualitative.[1]Proper screening for carriers in child bearing age and in children helps in early detection of heterozygote state. Detailed investigation for the cause of anemia by the HPLC(high performance liquid chromatography) helps in early detection and proper management of the patient. WHO figures estimate that 5% of the world population is a carrier for Hemoglobinopathies[2] and of these, thalassemia is most common for morbidity.

II.Materials and Method:

This is a retrospective cross-sectional study conducted in pathology laboratory of Dr. Vasantrao Pawar Medical College, Nashik, Maharashtra during the period of January 2022 –December 2022. Patients workup for clinical history like anemia, generalized weakness, fever, splenomegaly and family history were noted. Patients with a history of recent blood transfusion and inadequate sample were excluded from the study. Under all aseptic precautions, 5 ml of blood was collected in EDTA vacutainer and haemogram with electrophoresis were performed. For electrophoresis the samples were stored at 4-8℃ and were analyzed in batches within one week. Red blood cell indices were obtained from cell counter- Sysmex-XN550 the same was used for HPLC. The samples were run on instrument manufactured by Bio-Rad Laboratories. The instrument utilizes the principle of HPLC.An HbA2F calibrator and two levels of control were analyzed at the beginning of each run.
Hemoglobin(Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were seen. Blood smear examiantion done by Fields staining were studied to look for ancillary findings like anisopoikilocytosis, polychromasia, target cells, sickle cells and nucleated RBCs.

III. RESULTS:

A total of 150 cases were studied. Twenty One(21%) were found positive for abnormal Hemoglobins and is illustrated in"Table-1". There were 54 (36%) males and 73 (48.66%) females as seen in "Table-2".

(Table:1) Hemoglobinopathies diagnosed by HPLC:

Diagnoses	No of cases	%
Normal	119	79%
Beta thalassemia trait	14	9.33%
Beta thalassemia major	1	0.66%
Sickle cell trait	3	2%
Sickle cell disease	10	6.66%
Double hetereozygous	2	1.33%
Alpha chain variant	1	0.66%

(Table-2) Gender wise distribution:

Diagnosis	Male (%)	Female (%)	Total (%)
Normal	54(36%)	73(48.66%)	127
Beta thalassemia trait	3(2%)	11(2.03%)	14
Beta thalassemia major	-	1(0.66%)	1
Sickle cell trait	1(0.66%)	3(2%)	4
Sickle cell disease	2(1.33%)	6(4%)	8
Double hetereozygous	-	3(2%)	3
Alpha chain variant	-	1(0.66%)	1

The age group were ranged from 10 days to 70 years with a maximum number of patients in the age group 0-10 years is illustrated in "Table-3". Findings obtained from peripheral smear examination, family history, and relevant clinical signs and symptoms is suggestive of various hemoglobinopathies. Mean corpuscular volume, Mean corpuscular hemoglobin and Mean corpuscular hemoglobin concentration of various hemoglobinopathies are illustrated in "Table-4".

(Table-3) Age wise distribution of all positive cases:

Age (years)	Beta thalassemia trait	Beta thalassemia major	Sickle cell trait	Sickle cell disease	Double hetereozygous	Total
0-10	6	1	-	3	3	-
11-20	1	-	-	4	-	-
21-30	3	-	3	1	-	1
31-40	3	-	1	-	-	-
41-50	-	--	-	-	-	-
>50	1	-	-	-	-	-
total						

(Table-4) Complete blood count in overall study population:

Diagnosis	Hb(gm/dl) Mean SD	Rbc($x10^{6}$) Mean SD	MCV(fl) Mean SD	MCH(pg) Mean SD	MCHC(g/dl) Mean SD	RDW(%) Mean SD
Normal	7.0 ±3.2	3.5±1.2	68.91	22.7±6.3	31.2±4	18.3±4.2
Beta thalassemia trait	8.9± 2.4	4.7±1.0	60.9±7.3	19.3±2.44	31.4±1.0	17.2±3.0
Beta thalassemia major	5.0 ± 0.11	2.8 ± 0.4	63 ± 7.3	19.0 ± 2.6	31.0 ±1.2	22 ± 4

Sickle cell trait	7.5 ± 2.5	3.2 ± 1.8	72.2 ± 20	72.0 ± 1.8	31.2 ± 2.2	18.5 ± 5.3
Sickle cell disease	77.0± 1.9	2.5 ± 1	80 ± 11	82 ± 10	32 ± 2.0	20 ± 5.1
Double hetereozygous	8.2 ± 4.7	3.8 ± 2.5	71± 3.5	71.5 ±3.5	33.5 ± 5.0	20.1 ± 1.8

The figure below shows the normal HPLC(high performance liquid chromatography report "Figure-1".

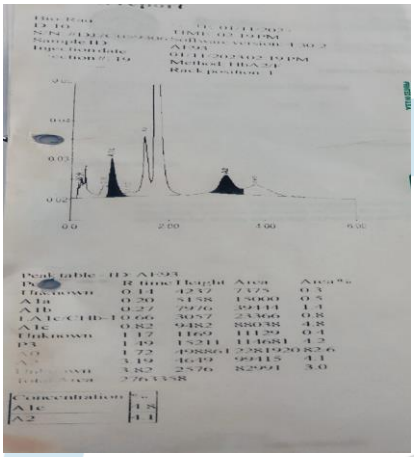


Figure:1 Normal HPLC Report

Of these prevalence of beta thalassemia trait is(9.33%). 21% cases displayed abnormal hemoglobin fractions on HPLC. The major abnormality observed was of high Hb A2. A cut-off of over 3.9% was taken for diagnosis of beta thalassemia trait[3]. A total of 14 cases (9.33%) of beta thalassemia trait were diagnosed as seen in "Figure-2". The retention time for Hb A2 was 3.19 minutes. Predominant peripheral blood findings were microcytosis and hypochromia. There were 1(0.66%) cases of beta thalassemia major. Hb F levels were raised with a variable reduction in Hb A.

Cases diagnosed with thalassemia major presented within the first
two years of life as illustrated in "Figure-3". Marked anemia, anisopoikilocytosis, microcytic hypochromic blood picture with many nucleated RBCs dominated the blood picture. All the cases had Hb F more than 90%. Two year old male with absence of beta thalassemia trait and slight raise in p3 suggest degradation or alpha chain variant.

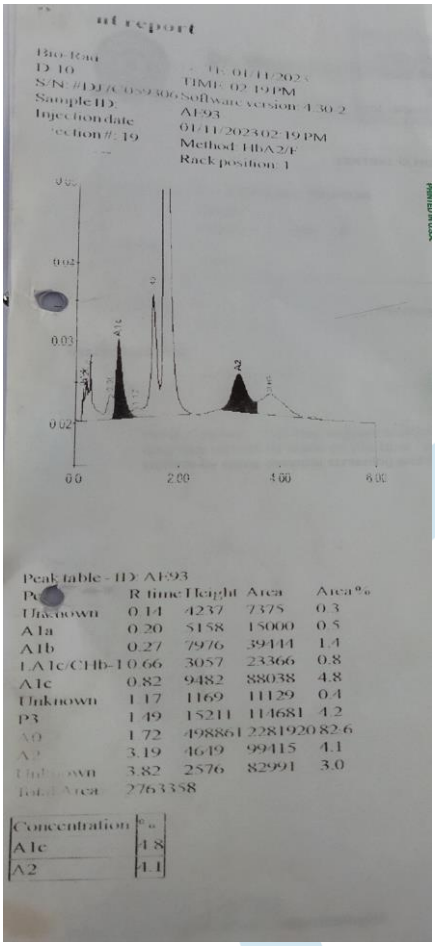


Figure:2 Beta thalassemia minor

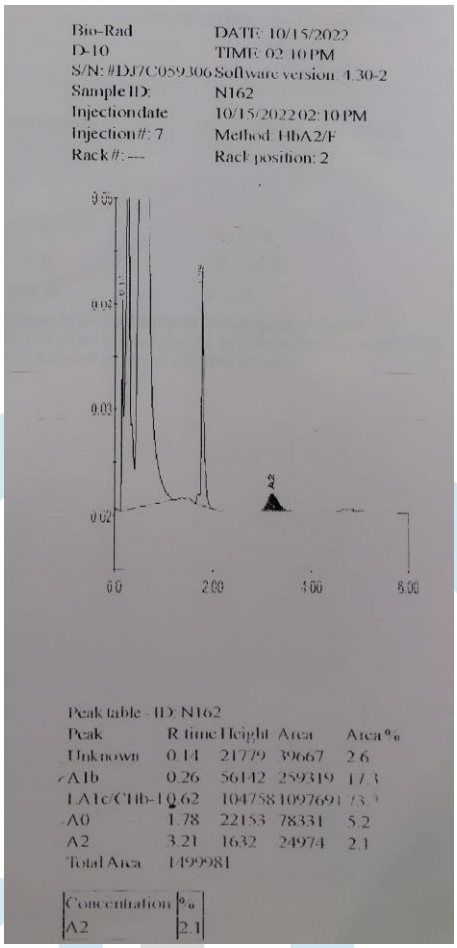
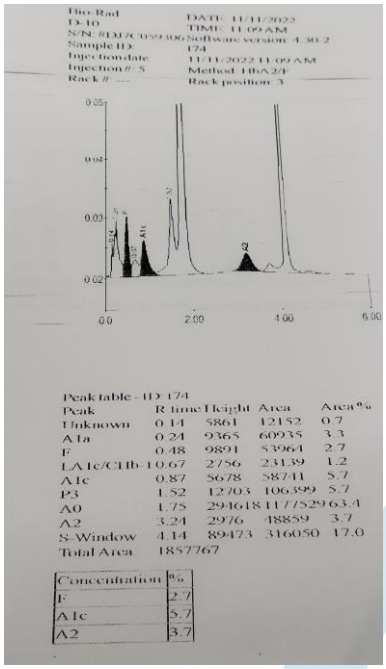


Figure:3 Beta thalassemia homozygous or double heterozygous for beta thalassemia and delta beta thalassemia.

Hb S homozygous (1 case) presented with a variant S-Window
of 59.2% test was positive in “Figure-4”. Hb was 7.2 gm/dL with target cells and few irreversibly sickled cells in the peripheral smear. Hb F was raised to 17.7%. Three cases had high Hb A2 along with high Hb S, MCHC blood picture with target cells. These were diagnosed as double
heterozygous for Hb S-beta thalassemia trait . Three cases of sickle cell trait were found with normocytic normochromic anemia “Figure-5”.

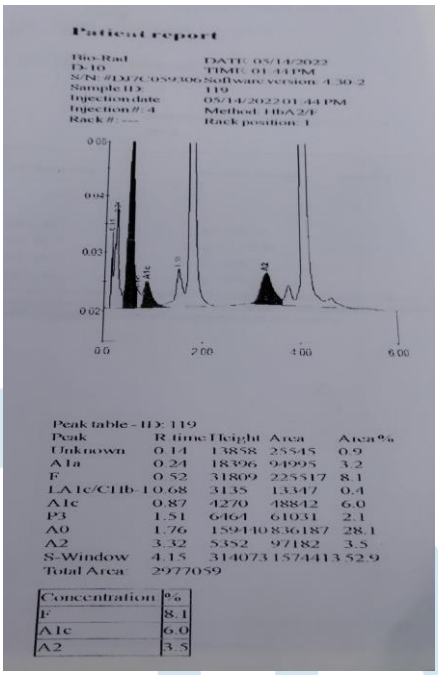


Peak table - ID: 174

Peak	R time	Height	Area	Area%
Unknown	0.14	5861	12152	0.7
A1a	0.24	9365	60935	3.3
F	0.48	9891	53964	2.7
LA1c/C1Ib	0.67	2756	23139	1.2
A1c	0.87	5678	58741	5.7
P3	1.52	12703	106399	5.7
A0	1.75	294618	1177529	63.4
A2	3.24	2976	48859	3.7
S-Window	4.14	89473	316050	17.0
Total Area			1857767	

Concentration	%
F	2.7
A1c	5.7
A2	3.7

Figure:4 HbS Heterozygous Syndromes



Peak table - ID: 119

Peak	R time	Height	Area	Area%
Unknown	0.14	13858	25545	0.9
A1a	0.24	18396	94995	3.2
F	0.52	31809	225517	8.1
LA1c/C1Ib	0.68	3135	13347	0.4
A1c	0.87	4270	48842	6.0
P3	1.51	6461	61031	2.1
A0	1.76	159410	836187	28.1
A2	3.32	5352	97182	3.5
S-Window	4.15	314073	1574413	52.9
Total Area			2977059	

Concentration	%
F	8.1
A1c	6.0
A2	3.5

Figure:5 HbS Homozygous Syndromes

Four month old male with 90.5% foetal hemoglobin suggested beta-thalassemia homozygous and delta beta thalassemia. A possibility of hereditary persistence of fetal hemoglobin was raised in such cases with a recommendation for molecular confirmation. Hb Lepore constituted one case. Hb A2 was raised to 12.1% with mild anemia. “Figure-6”.

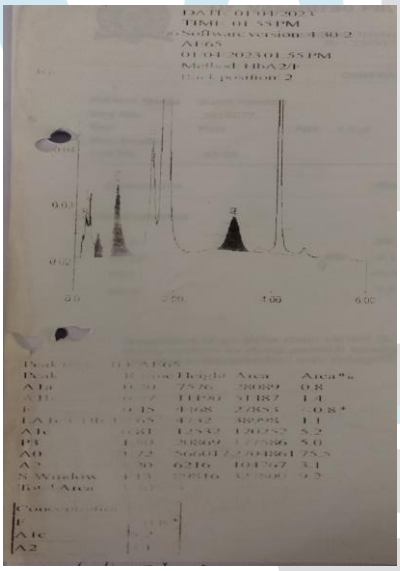


Figure:6 Suggestive of alpha chain variant, most likely Hb Russ or HBG- Walmanalo

IV. CONCLUSION:

To conclude, HPLC is an ideal method for routine diagnosis of hemoglobinopathies. In a country where nutritional deficiency is considered the most common we should also considered the presence of abnormal hemoglobin's type. Continuous awareness programs, mass screening of the population especially during childbearing age and school going children will help in early detection of heterozygous states. This can in turn with proper genetic counseling help in reducing the morbidity and mortality.

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