Prevalence of Thalassemia and Hemoglobin Variants by HPLC in Out Patient Laboratory

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Abstract: Background: Inherited disorders of hemoglobin are common and their identification is essential epidemiologically and help to prevent serious hemoglobin disorder. Method: study was conducted in patients attending outpatient laboratory, Civil Hospital, Ahmedabad. Total 466 cases were scrutinized on basis of Mentzer's index (MCV /RBC count < 13) and sent for HPLC at Red Crosssociety, Ahmedabad. Result: The Out of these, abnormal hemoglobin fraction was found in 107(22.90%) cases. Beta thalassemia trait was found predominantly. Other Hb variants like HbS, HbE, Hb D Punjab, Hb F and some double heterozygous Hb variants like Hb S-α thalassemia, Hb D-β thalassemias were also observed. Conclusion and Interpretation: Beta thalassemia trait was found to be predominant. [Dhruti P NJIRM 2017; 8(2):23-27]

Key Words: HPLC (high performance liquid chromatography), Hemoglobinopathies, Hb Variants, Thalassemia

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Introduction: Abnormalities of haemoglobin synthesis are common inherited disorders. These disorders can be quantitative (thalassemia syndrome) or qualitative (variant HbS).[1] Of these, thalassemia syndromes particularly β thalassemia major and certain α thalassemia are serious and major cause of morbidity. Accurate and timely detection of various haemoglobin variants including β thalassemia heterozygous can prevent the occurrence of more serious disorders like thalassemia major in newborns. Potential interaction between various Hb variants in the offspring. Double heterozygous state between certain variants can also lead to haematological defect.The use of cation exchange HPLC to separate and quantify various normal and abnormal haemoglobin fraction has been recommended.[2-5] It is highly sensitive , specific , fast but more expensive method for diagnosis.

Majority of centres in India use conventional methods for the diagnosis of haemoglobinopathies that includes clinical and family history, red cell indices, CBC, HbF estimation, sickling test and Hb electrophoresis. However, these methods have limitations include identification of Hb variants with same electrophoretic mobility, diagnosis of HbS traits where low quantity of HbS is associated with negative sickling test and diagnosing compound heterozygous states (HbS- Beta thalassemia, HbS-HbD disease).

Methods: The present study was carried out at outpatient pathology laboratory, Civil Hospital , Ahmedabad for two month. A total 5298 patients attended the laboratory. Among these, 466 cases were selected on basis of Mentzer's index (MCV/RBC count < 13) and sent for HPLC at Red cross society , Ahmedabad. Specimens were drawn into K_3 EDTA tubes with BD(Becton Dickinson) vacutainer system. After collection, the samples were stored at 2-8^{0}c and tested within week of. CBC with red cell indices and peripheral blood examinations were done in all cases. The samples were assessed by Bio-Rad Variant utilising the principle of HPLC.

Results: Out of these 466 cases, 107(22.90%) cases displayed abnormal Hb fraction. Table 1 and 2 shows males (63) are more commonly affected. The major abnormality observed was high Hb-A_2 (95). Cut off value over 3.9% was taken for diagnosis of β thalassemia trait.[7] A total of 95(20.38%) cases of β thalassemia trait were diagnosed.

Table 1 shows spectrum of haemoglobinopathies (n=107)

Diagnosis	No Of Cases	Hb Variant	Retention Time Minute
Thalassemia Minor	95(20.38%)	HbA2 (3.9-5.9%)	3.64-3.68
Thalassemia Major	1(0.21%)	HbA2 (2.1%) HbF (99.2%) HbA (0.7%)	3.65 1.18 2.50
Sickle Cell Disease	1(0.21%)	HbS (75.3%) Hb F (13.0%)	4.43 1.16
Sickle Cell Trait	2(0.42%)	HbS (35.2-37.5%)	4.49-4.51
Hbs-B Thalassemia Trait	1(0.21%)	HbS (68.4%)	4.46

		HbA2 (5.8%) HbF (15.4%)	3.44 1.16
Hbd Punjab	3(0.63%)	HbD (28.5-28.9%)	4.08-4.12
Hbd Punjab- B Thalassemia Trait	1(0.21%)	HbD (77.8%) HbF (7.5%)	4.08 1.16
Hbe TRAIT	1(0.21%)	HbE (34.1%)	3.65
Hereditary Persistent Fetal Hemoglobin	2(0.42%)	HbF (86.2%) HbA2 (2.68%)	1.18 3.65
		HbF (23.5%) HbA2 (1.9%)	1.17 3.61

The major abnormality observed was high Hb-A_2(95).

Table 2 shows sexwise distribution of hemoglobinopathies

Diagnosis	Male	Female	Total
Thalassemia Minor	56	39	95
Thalassemia Major	01	00	01
Sickle Cell Disease	01	00	01
Sickle Cell Trait	02	00	02
Hbs-B Thalassemia Trait	00	01	01
Hbd Punjab	02	01	03
Hbd Punjab– B Thalassemia Trait	00	01	01
Hbe Trait	00	01	01
Hereditary Persistent Fetal Hemoglobin	01	01	02
Total	63	44	107

Males are more commonly affected.

Figure 1- showing A) Thalassemia minor B) Thalassemia major

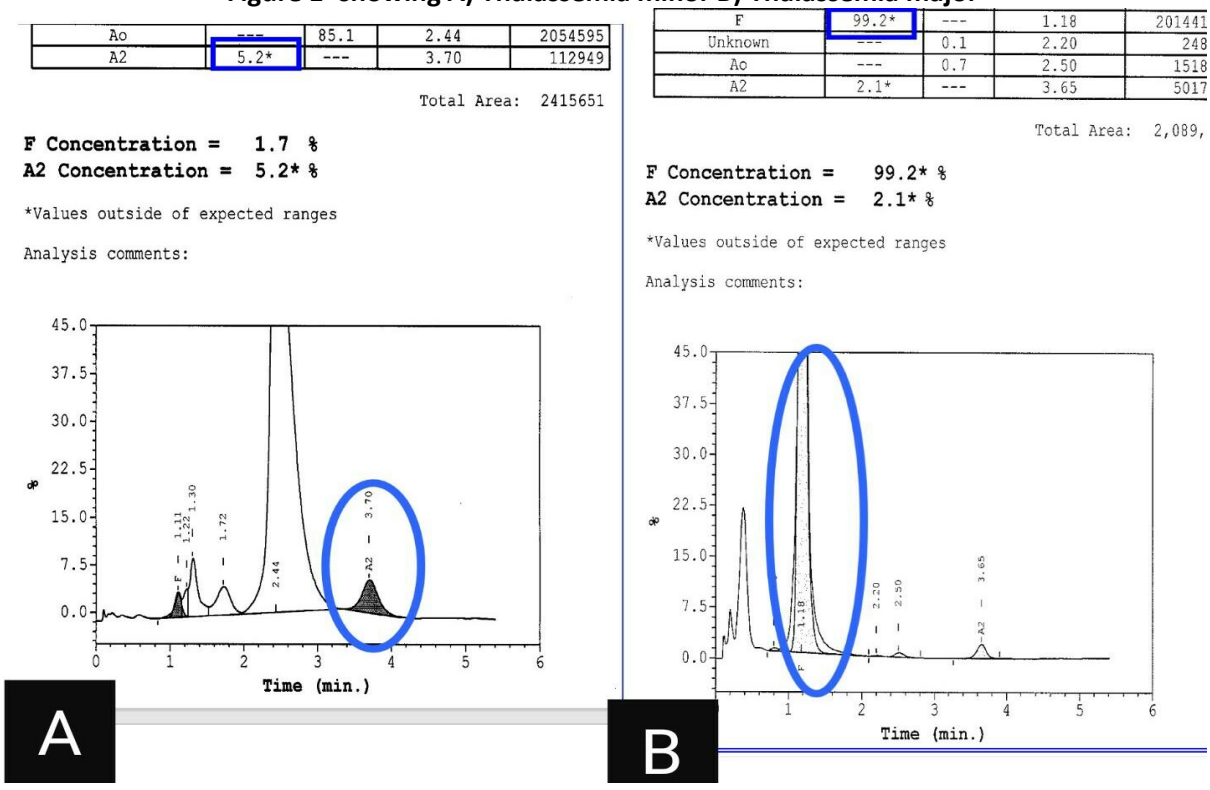
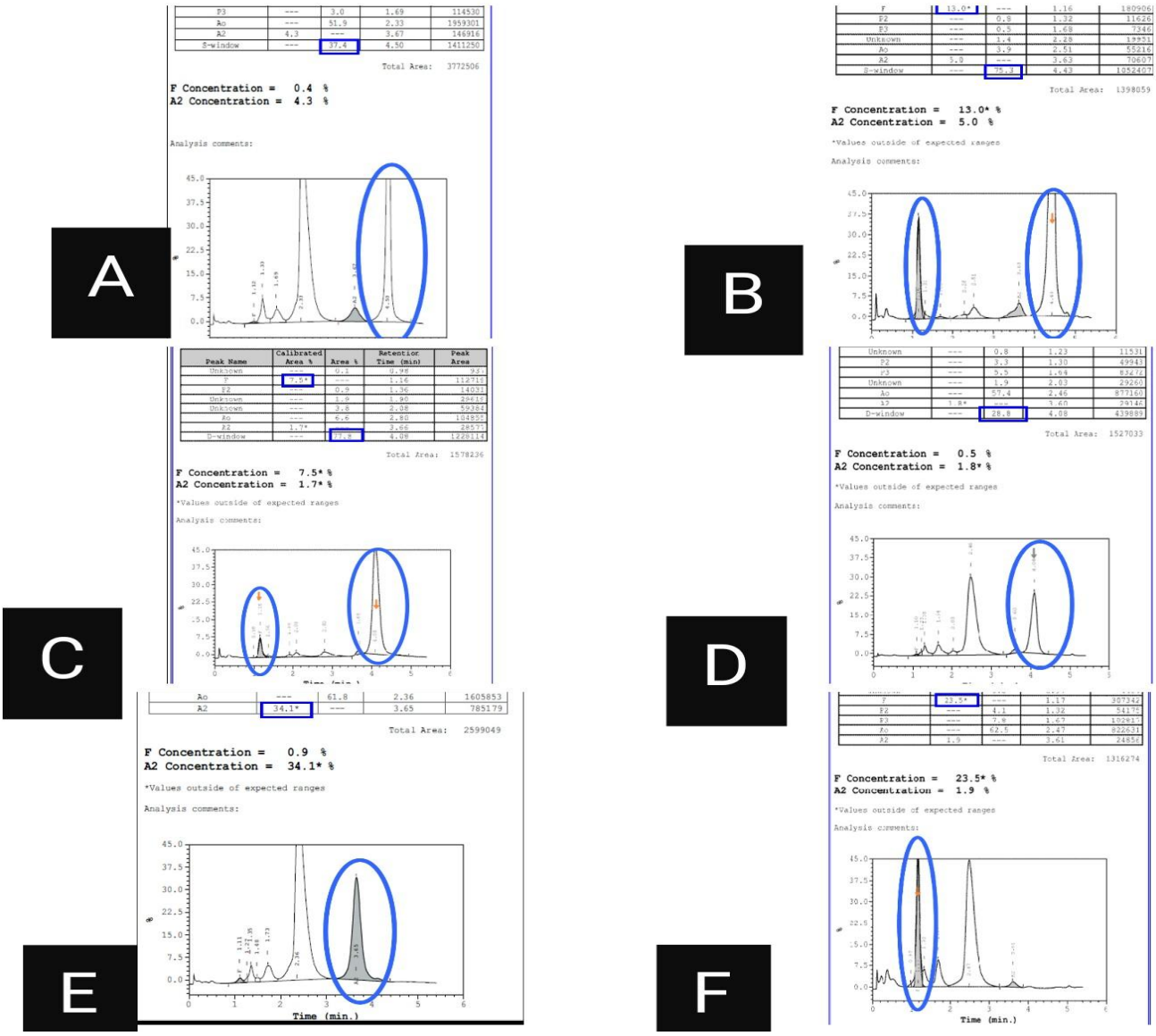


Figure 2- showing HPLC graphs in A) Sickle cell trait B) Sickle cell disease C) HbD Punjab- β thalassemia trait D) HbD Punjab E) HbE trait F) Hereditary Persistent Fetal Hemoglobin



Discussion: The laboratory diagnosis of hemoglobinopathies and thalassemias is essential for 1.confirmation of sickling disorders and thalassemias 2. find out the cause of underlying hematologic abnormality (such as anaemia, microcytosis or polycythemia), 3.neonatal screening 4. to identify the abnormality in the pre symptomatic phase 5.to predict serious disorders of the globin-chain synthesis in the foetus and offer the option of termination of pregnancy6.to permit genetic counselling of prospective parents[7].

Our study predominantly included patients attending Out Patient Pathology Laboratory , Civil Hospital , Ahmedabad , Gujarat. A total of 22.74 % Hb variants were detected. β thalassemia trait formed the largest subgroup of abnormal group(20.38%), and β thalassemia major was seen in one patient(0.21%). The low incidence of homozygous state of the disease may be either to decrease incidence due to effective prenatal screening or may be due to under reporting.

Majority of β thalassemia trait (22.38%) were reported in 21-30 years of age group. The high incidence of traits underscores the need for antenatal screening for prevention of thalassemia major in the offspring. Conditions with borderline HbA2 need careful interpretation . Iron deficiency may lead to low HbA2 and hence may mask thalassemia trait , whereas B12/folate deficiency may lead to slightly raised HbA2 leading to false diagnosis of a trait . Careful evaluation with indices with iron profile will usually help in such cases. Similarly milder forms of thalassemia or co-

inheritance of delta thalassemia may lead to border linen HbA2 levels. Genetic testing should be advised in such cases for conclusive opinion.

HbS homozygous presented as S window of 75.3% with HbF 13% in one case (0.21%). Value of HbF are generally raised in parts of Central India and Orissa. Sickling test was positive. One case of double heterozygous for Hb S-β thalassemia trait showed S window of 25.3% and Hb F> 5%. In β thalassemia trait Hb F <2%. HbS level was reduced in association with β thalassemia with raised RBC (Red Blood Cell) Count, low MCV(Mean Corpuscular Volume) and low MCH(Mean Corpuscular Hemoglobin).

HbD Punjab tends to have a normal phenotypic presentation. There is a mutation in the β chain at β121 Glu→Gln (GAA-CAA).[8] HbD Punjab was observed in four (0.84%) cases . On HPLC it elutes in D window 28.8 %, separate from HbS peak and Hb F 0.5% .One case of double heterozygous for HbD and β thalassemia trait showed D window of 77.8% and Hb F 7%. Patient tend to have mild anemia and are asymptomatic. Molecular diagnosis is required for final confirmation.

HbE results from a β chain mutation (β26 Glu→Lys)[9] and tends to elute in A2 window on HPLC. HbE homozygous individuals are normal , HbE levels are usually 30% which elutes in the HbA2 window . The percentage of HbE may be low in case of co-existence iron deficiency and α thalassemia mutation. The possibility of α thalassemia , normal A2 β thalassemia or other hemoglobinopathies that elute with similar retention values cannot be ruled out by HPLC. A desclaimer should always accompany reports.[10]

2 cases had isolated HbF elevation with normal blood counts. A possibility of hereditary persistence of foetal haemoglobin was raised in such cases with a recommendation of molecular confirmation. And both of two diagnosed as of hereditary persistence of foetal haemoglobin in further evaluation.

The Bio-Rad HPLC systems are automated cation exchange HPLC instruments that have been used to quantify HbA2, HbF, HbA along with screening haemoglobin variants like HbS, HbD, HbE and HbC in a single, highly reproducible system, making it an excellent technology to screen for haemoglobin variants and hemoglobinopathies along with thalassemia. With the integration of proper algorithm involving retention time, % haemoglobin, and RBC indices, a clinical laboratory is capable of identifying about 75% 0f common variants encountered without the need for confirmatory studied such as alkaline and acid electrophoresis.

Our study had 20.38% β thalassemia trait. Early detection of these traits will prevent occurrence of thalassemia major in the offspring. More importantly , identification of the common haemoglobin variants (i.e. HbD Punjab, HbE and β thalassemia) in combination with HbS lead to clinically significant sickling disorder which can be quickly and accurately accomplished by HPLC without the need for confirmation testing.[11]

The observations must be supplemented by haemogram findings, family/sibling studies, Hb electrophoresis , other confirmatory techniques and molecular studies based on HPLC findings and on a case to case basis.[10]

Conclusion: The simplicity of sample preparation , accurate quantification of haemoglobin concentrations combined with complete automation make HPLC an ideal methodology for routine diagnosis of Hb disorders.

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