ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 7, Issue – 4, August – 2022 , Page No. : 33 – 38

Interesting Cases of Hemoglobinopathies Detected on High Performance Liquid Chromatography (HPLC) - Report Of 5 Cases.

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Citation this Article: Dr. Kalyani Raman Reddy, Dr. Archana M. Joshi, Dr. Anjali D. Patrikar, " Interesting Cases of Hemoglobinopathies Detected on High Performance Liquid Chromatography (HPLC) - Report Of 5 Cases", IJMSIR- August - 2022, Vol – 7, Issue - 4, P. No. 33 – 38.

Type of Publication: Case Report

Conflicts of Interest: Nil

Abstract

Hemoglobinopathies are one of the major public health problems in Maharashtra, India. Their prevalence shows regional and ethnic variations. We are hereby presenting case report of interesting patterns of hemoglobinopathies detected by HPLC by BIORAD variant using beta thalassemia short program in and around Nagpur, Maharashtra, India. A total of 400 cases were studied at Pathology department at tertiary care center.

Laboratory records of patients were screened and analyzed, 54 (13.5%) were found positive for abnormal hemoglobinopathies. Among which prevalence of sickle trait with 9.75% followed by sickle cell disease, beta thalassemia trait, sickle-thalassemia double heterozygous in decreasing order. Other rare variants such as HbD Punjab and Hb Hope were also found. Continuous awareness programs, mass screening of the population is important for preventing development of severe diseases in newborn. This can be achieved by proper genetic counselling and early prenatal diagnosis which will help in reducing the morbidity and mortality.

Keyword: Haemo globinopathy, High performance liquid chromatography.

Introduction

The hemoglobinopathies are a group of disorders inherited through families in which there is abnormal production or the structure of haemoglobin molecules. Hemoglobinopathies are one of the major public health problems in the state of Maharashtra, India.[1] Hemoglobinopathies can be either quantitative or qualitative.[2] WHO figures estimate that 5% of the world population is a carrier for hemoglobinopathies.[3]

Most of the cases of hemoglobinopathies in India are diagnosed by conventional methods, which include clinical and family history, red cell indices, complete blood counts, Haemoglobin (Hb) F estimation, sickling

test, and Hb electrophoresis.[1] These conventional methods have limitations in the identification of Hb variants with same electrophoretic mobility and diagnosing certain compound heterozygous states.[9] To overcome this problem, cation exchange HPLC is used to separate and estimate various normal and abnormal Hb fractions.[4] It offers a definitive tool for early and accurate detection of hemoglobinopathies, thereby aiding in their prevention and management.[5] HPLC is one of the most important tool for either screening or confirmation of hemoglobinopathies with relatively high sensitivity and specificity.[6] HPLC and its correlation with complete blood count and family studies can aid the diagnosis of majority of hemoglobinopathies. The merits of HPLC are requirement of small quantity of sample, less TAT, speedy diagnosis and accurate categorization of Hb S, Hb A2, Hb F. [7,8] Presenting here few interesting cases of hemoglobinopathies detected by HPLC on samples received in and around Nagpur in a study carried out at tertiary care hospital.

Material and methods

400 cases were observed and analysed at Pathology department by HPLC, BIORAD variant using beta thalassemia short program. Patients with a history of recent blood transfusion and inadequate sample were excluded from the study. An HbA2F calibrator and two levels of control were analyzed at the beginning of each run. Based on retention time and proportion of Hb variants, different hemoglobinopathies were diagnosed and their prevalence was analyzed and correlated with RBC parameters and clinical history. Among these we are presenting 5 interesting cases showing the spectrum of hemoglobinopathies in our region.

Case reports

1. A 31 yr old male came for routine screening. Haemoglobin, PCV were reduced and RDW was raised. MCV, MCH, MCHC were within normal limits. HPLC findings were,

HbF -14.5%, A_0- 3.5%, A_2-1.8%, SWind- 77.4% (Fig.1)

Impression : “SS” Pattern

2. A 55 yr old male came for routine screening. Haemoglobin, PCV and RBC were slightly reduced. RDW was raised(23.6). MCV, MCH, MCHC were within normal limits. HPLC findings were, HbF-16.3%, A_0- 22.6%, A_2-4.5%, SWind- 52.1% (Fig.2)

Impression : Sickle thalassemia double heterozygous.

3. A 25yr old female came for routine ANC screening. All red cell indices were within normal limits. HPLC findings were, HbF-2.6%, A_0- 81.3%, A_2-5.1% (Fig.3)

Impression : β thalassemia trait.

4. A 58 yr old female came for routine examination. All CBC parameters were normal.HPLC findings were, HbF-<0.8%, A_0- 50.8%, A_2-2.2%, Unknown peak 37.3% at 3.87min retention time (Fig.4)

Impression : HbD Punjab Heterozygous.

5. A 34yr old female came for routine examination. All CBC parameters were normal with slightly decreased haemoglobin and PCV. No history of blood transfusion. Blood sugars were also normal.

HPLC findings were, HbF-2.9%, A_{1C}- 53.1%, A_0- 40.8 %, A_2-1.8% (Fig.5)

Comments: Considering raised HbA1C and normal sugar levels and no history of BT. HbF and Hb Hope can elute in positions of HbA1C. Suspicious of Hb Hope. Confirmed by capillary electrophoresis and diagnosis given as Hb Hope.

Results

Laboratory records of total 400 patients screened for suspected hemoglobinopathies were analyzed. Fifty four (13.5%) were found positive for abnormal Hbs. There were 107 (61%) males and 68 (39%) females. The age

group ranged from 27 days to 80 years with a maximum number of patients in the age group 20-30 years Criteria for suspecting hemoglobinopathy in these cases included: Results of screening tests such as various discriminant functions so obtained on hematology cell counters, findings obtained from peripheral smear examination, family history, and relevant clinical signs and symptoms suggestive of hemoglobinopathy. The different hemoglobinopathies found are as shown in Table2.2. We found that a maximum number of patients were of sickle cell trait with the prevalence of 9.The patients of sickle cell disease had Hb S 70-90%, Hb F 10-30% and Hb A 0-10% were diagnosed as sickle cell disease. Haemoglobin and packed cell volume were reduced and red cell distribution width was raised and sickling test was positive. MCV, MCH and MCHC were within normal limits. In case of sickle thalassemia double heterozygous, S-window was 52.1% and HbA2 was 4.5%, his haemoglobin, packed cell volume and RBC count was slightly reduced and RDW was raised. Beta thalassemia trait was diagnosedbased on high levels of HbA2 (4-8%). These patient came for routine ANC examination and all red cell indices were within normal limits. Fetal Hb was not raised. HbA2 of 3.5-3.9% was considered as borderline. These cases were advised iron status studies and repeat HbA2 levels after iron therapy.

We found three case of Hb D Punjab heterozygous. This patient had mild hemolytic anemia. There was a unknown band of 37.3 % in retention time of

3.87 min. One case each of Hb E trait and disease was identified. Hb E trait had Hb A0 40.8%,HbA2 1.8% and A1C was 53.1%. All CBC parameters were normal with slightly decreased haemoglobin and packed cell volume. There was no history of blood transfusion and sugar levelswere normal.

Discussion

Potential interactions between various Hb variants in heterozygous state may lead to serious homozygous Hb variants in the offspring. Double heterozygous states between certain variants can also lead to hematological defects. The 5 cases presented above shows that the correlation between HPLC findings and red cell indices helps in accurate diagnosis We found that the maximum numbers of cases were of sickle cell trait (9.75%). The high incidence of sickle cell trait call for the need of antenatal screening and screening of marriageable age groups. This will help in prevention of sickle cell disease in off springs. This trend is probably started in educated and affording population. This could be the one of reason for detecting less number of cases of sickle cell disease in our study group. We think that antenatal screening or screening of higher secondary school children to detect hemoglobinopathies will definitely help in drastically reducing the incidence of the sickle cell disease and thalassemia major.

Hb S homozygous presents as an S-Window with abnormal haemoglobin ranging from 70-90% and increased values of Hb F. Clinical presentation in sickle cell anemia and its various heterozygous states varies according to severity of disease. It may present as mild anemia to severe complications like Vaso occlusive crisis, aplastic crisis, splenic sequestration, acute chest syndrome; Pulmonary hypertension, avascular necrosis, stroke resulting in varying degrees of neurological deficit; Renal, eye, cardiac involvement, leg ulcers, Hand-foot syndrome etc. During childhood and adolescence, SCD is associated with growth retardation, delayed sexual maturation, and being underweight. In our first case, patient was asymptomatic, undiagnosed came for routine screening with reduced Hb, PCV and RBc count and raised RDW. The diagnosis was given as

sickle cell disease by correlating solubility test, Hb electrophoresis, red cell indices and HPLC findings which showed S window of 77.4%, along with raised Hb F i.e. 14.4%.

β thalassemia heterozygosity known as β thalassemia trait. Usually it is clinically silent phenotype hence called thalassemia minor. In our case, patient was asymptomatic and Hb, MCV, PCV were normal with raised RBC count. With this background and on HPLC HbA2 was 5.1% so the diagnosis was made as β thalassemia trait.

Presentation of Hb S - β Thalassemia double heterozygous state may be asymptomatic or may resemble Sickle cell disease. In our case Hb, PCV and RBCcount was slightly reduced with raised RDW. MCV, MCHC, MCH were within normal limits. On HPLC-

S window is seen with area of 52.1%, HbF 16.3% and percentage of Hb A2 around 4.5%. Therefore, diagnosis was given as sickle thalassemia double heterozygous.

One case of HbD Punjab was identified. This patient had mild hemolytic anemia. HbD Punjab is one of the common hemoglobinopathy in India with prevalence of 1-3% of populations of North-West India. Hb D was identified by a peak in the D window with a retention time of 3.87min. These were differentiated by from HbS peak(retention time 4.30-4.70min). Hb D Punjab can be inherited in heterozygous with HbA or homozygosis, both usually have no hematologicaly alterations although later one can occasionally develop mild to moderate hemolytic anemia. The association of Hb D with other haemoglobin such as HbS or thalassemia can also occur. However when HbD associates to HbS, the double heterozygous, the result is moderate to severe clinical manifestations similar to homozygosis of HbSS. So to prevent the inheritance, HbD detection and screening is very important.

Among the rarely encountered variants we have detected 1 case of Hb Hope. In CE-HPLC method, it may be mistaken as high HbA1c, as it comes in the same position as HbA1c. This variant is clinically silent. or may show mild anemia with normal retic count. Detection of this variant is very important because when combined with thalassemia or HbS, it gives rise to moderate to severe disease to be inherited in off springs as double heterozygous. In our case, patient was asymptomatic, no history of transfusion. Sugar levels were normal there was raised HbA1C around 53.1 on HPLC. So we performed repeat test, same findings were seen. So for confirmation capillary electrophoresis was done and diagnosis was given as Hb Hope.

Conclusions

Various hemoglobinopathies are detected by HPLC which remain undetected by routine electrophoresis as most of the patients are asymptomatic or present with non-specific symptoms, thus there is difficulty in exact diagnosis. Therefore, HPLC is an ideal method for routine diagnosis of certain hemoglobinopathies. Continuous awareness programs, mass screening of the population especially in childbearing age and school going children will help in early detection of heterozygous states, and is important for preventing development of severe diseases in newborn. This can be achieved by proper genetic counselling and early prenatal diagnosis which in turn will help in reducing the morbidity and mortality.

But we should also be aware of the limitations and problems associated with this method that is not easily available, cost effective and due to elution of variant haemoglobins within the same retention windows. With all these limitations, HPLC is an excellent and powerful diagnostic tool for the direct identification of haemoglobin variants with high degree of precision and

also in the quantitation of normal and abnormal haemoglobin fractions.

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Table 1: Manufacturer assigned windows for Bio-Rad variant high-performance liquidchromatography system

AnalyteName	Retention time (min)	Band (min)	Window(min)
F	1.15	0.15	1.00-1.30
P2	1.45	0.15	1.30-1.60
P3	1.75	0.15	1.60-1.90
AO	2.60	0.40	2.20-3.30
A2	3.83	0.15	3.68-3.98
D-window	4.05	0.07	3.98-4.12
S-window	4.27	0.15	4.12-4.42
C-window	5.03	0.15	4.88-5.18

Table 2.1 and 2.2: Spectrum of hemoglobinopathiesTable 2.1

Non hemoglobinopathies	Hemoglobin opathies	Total cases studied
346	54	400

Table 2.2. Number of cases and percentages according to haemoglobinopathies

Name	Number of cases	Percentage
Sickle cell trait	39	9.75
Sickle cell disease	8	2
Hb D Punjab	3	0.75
Beta thalassemia trait	2	0.50

Sickle thalassemia double heterozygous	1	0.25
Hb Hope	1	0.25

Figures with legends

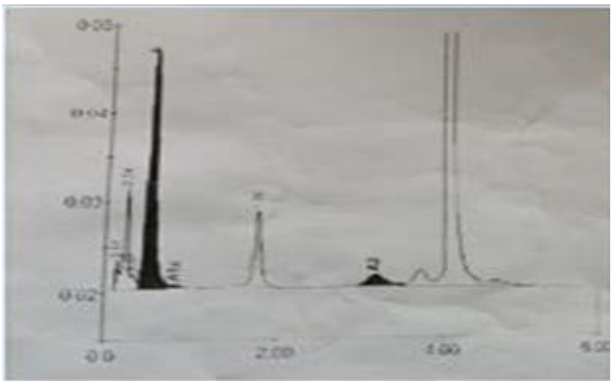

Figure 1: Sickle cell disease-HbF -14.5%, A_0- 3.5%, A_2- 1.8%, S Window- 77.4% (Fig.1)

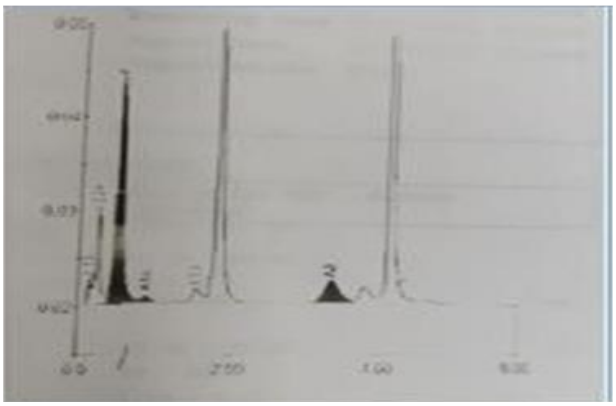

Figure 2: Sickle thalassemia double heterozygous- HbF- 16.3%, A_0- 22.6%, A_2-4.5%, S Window- 52.1%

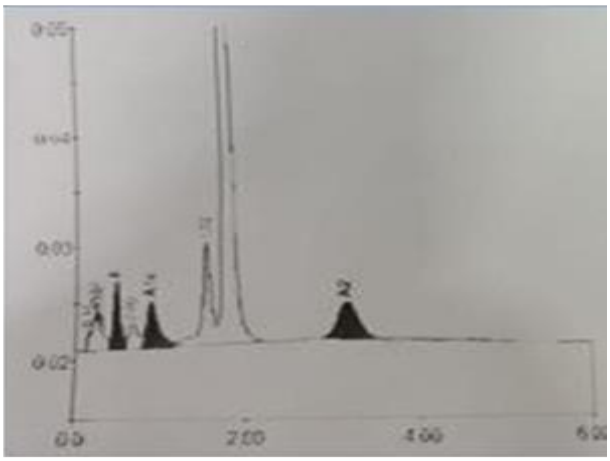

Figure 3: β thalassemia trait -HbF-2.6%, A_0- 81.3%, A_2- 5.1%

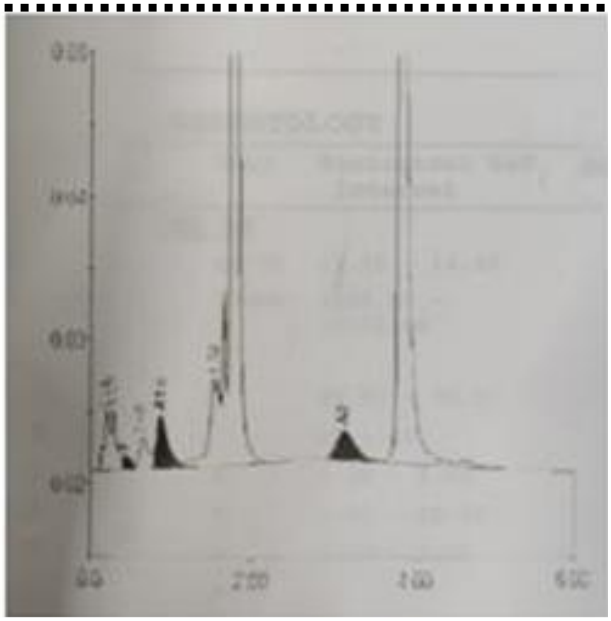

Figure 4: HbD Punjab Heterozygous -HbF-<0.8%, A_0- 50.8%, A_2-2.2%, Unknown peak 37.3% at 3.87 min retention time

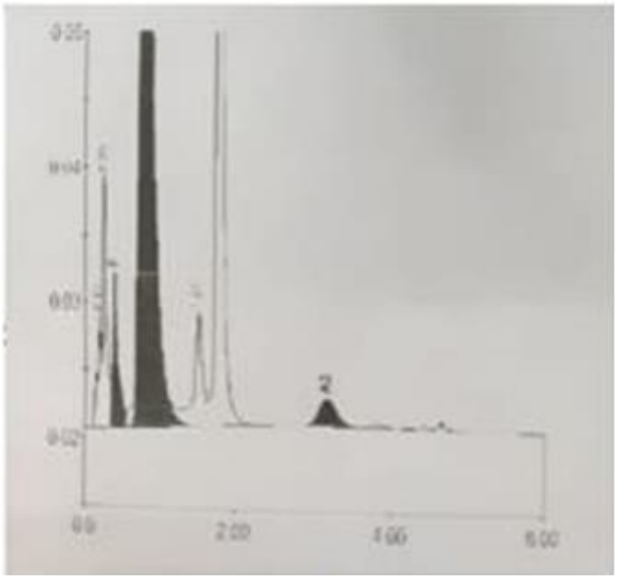

Figure 5: Hb hope- HbF-2.9%, A_1C- 53.1%, A_0- 40.8 %, A_2-1.8%