Original Research Article

HPLC profile of sickle cell disease in central India

Shweta P. Bijwe*

Department of Pathology, IGGMC, Nagpur, Maharashtra, India
*Corresponding author email: **dr.shwetabijwe@gmail.com**

International Archives of Integrated Medicine, Vol. 5, Issue 1, January, 2018.

Available online at http://iaimjournal.com/
ISSN: 2394-0026 (P) **ISSN: 2394-0034 (O)**
Received on: 21-12-2017 **Accepted on:** 28-12-2017
Source of support: Nil **Conflict of interest:** None declared.
How to cite this article: Shweta P. Bijwe. HPLC profile of sickle cell disease in central India. IAIM, 2018; 5(1): 36-41.

Abstract

Background: Various hemoglobinopathies are one of the major public health problems of India.
Aim: To study spectrum of sickle cell disease in Central India based on HPLC, to identify abnormal hemoglobin requiring counselling and PND and to monitor treatment modalities.
Material and methods: A retrospective study of total 5819 patients was done during January 2003 - December 2012, at RHDMC, IGGMC, Nagpur. Inclusion criteria were patients presenting with anemia, joint pain and jaundice, relatives on patients diagnosed with sickle cell disease and all antenatal cases. Tests performed in every case were hematological indices, Solubility test, Alkaline hemoglobin electrophoresis at PH- 8.9. All patients showing abnormal pattern on electrophoresis were subjected to HPLC on biorad variant.
Results: Total 2590 cases were identified with various hemoglobinopathies. Sickle cell trait was seen in 1172 cases (20.15%), homozygous sickle cell anemia was seen in 969 cases (16.65%), Hb S - β Thalassemia double heterozygous state in 222 cases (3.82%), HbS - Hereditary persistence of fetal haemoglobin in 82 cases (1.42%), Hb E trait in 59 cases (1.01%0, Hb D trait in 52 cases (0.9%), Hb J in 9 cases (0.17%), Homozygous Hb E and Homozygous Hb D in 7 (0.12%0 cases each, Sickle cell - Hb D double heterozygous state in 6 cases (0.1%), Sickle cell - Hb E double heterozygous state in 4 cases (0.07%) and Hb Q in 1 case (0.02%).
Conclusion: Various hemoglobinopathies have different clinical profile, thus their identification is important. Antenatal screening for hemoglobinopathies is important for preventing development of severe disease in newborn, which can be achieved by counselling and prenatal diagnosis in select cases. Region wise documentation of prevalence of various hemoglobinopathies is essential for epidemiological studies. HPLC is best tool for screening of hemoglobinopathies and Thalassemia. However, DNA studies in select cases are essential for confirmation.

Key words

HPLC, Hemoglobinopathies, Hemoglobin variants, Sickle cell disease.

Introduction

Abnormalities of hemoglobin (Hb) synthesis are among the most common inherited disorders of man and can be qualitative (variant Hbs) or quantitative (thalassemia syndrome) [1]. WHO figures estimate that 5% of the world population is carrier for Hb disorders [2]. Sickle cell disease and hemoglobinopathies are prevalent in Central India region. Presentation of hemoglobinopathies can vary from mild anemia to severe complications which can lead to disability and mortality.

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 use of cation-exchange high performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal Hb fractions has been increasing [3]. It offers a reliable tool for early, accurate detection; thereby aiding in prevention and management of various hemoglobinopathies [4].

Majority of the centers in India use conventional methods for diagnosis of hemoglobinopathies, like taking clinical and family history, red cell indices, complete blood counts (CBC), HbA2, HbF estimation, sickling test, and Hb electrophoresis. The limitations of these methods include identification of Hb variants with same electrophoretic mobility as in S/D/G/Q/Lepore and A2/E/C and diagnosing certain compound heterozygous states (Hb S + β thal, Hb S + Hb D, Hb D + Hb E, Hb E + β thal, Hb D + β thal) [5].

Materials and methods

This study was conducted in the Department of Pathology, Regional hemoglobinopathy detection and management centre, IGGMC, Nagpur. Total 5819 patients were studied during January 2003 - December 2012.

Inclusion criteria

- Patients presenting with anemia, joint pain and jaundice.
- Relatives of patients diagnosed with sickle cell disease.
- All antenatal cases.

A detailed clinical history and family history were obtained. History of blood transfusion, if present, was noted.

Tests performed in every case-

- Hematological indices
- Solubility test
- Alkaline hemoglobin electrophoresis at PH 8.9.

All patients showing abnormal pattern on electrophoresis were subjected to HPLC. Blood samples were collected in ethylene diamine tetrachloride acetate vials and analyzed with Sysmex automated cell counter for complete blood counts. High performance liquid chromatography was performed on Biorad Variant. HPLC is based on exchange of charged groups on an ion exchange material for charged groups on Hb molecule. Hbs are identified on the basis of retention time that is defined as the time in minutes from sample injection to the maximum point of the elution peak. Quantification of the Hbs is done by determining the area under the corresponding peak in the elution profile. Retention times are used to define the manufacturer assigned windows of chromatogram [6].

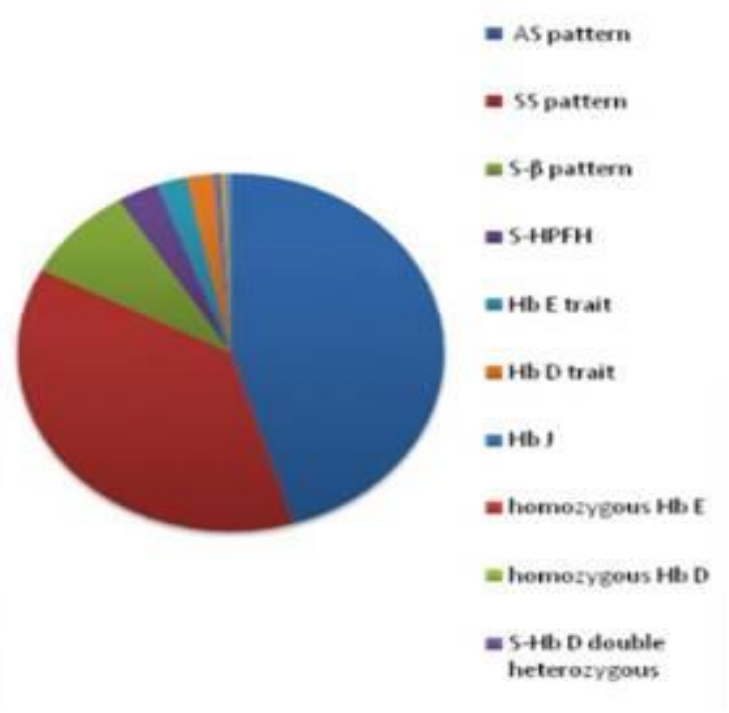
Results

A total of 5819 cases were studied. Of these, 2590 cases displayed abnormal hemoglobin fractions on HPLC.

Figure – 1 shows abnormal hemoglobin fractions on HPLC.

Figure – 1: Abnormal hemoglobin fractions on HPLC.

Hbpathy	%
AS pattern	20.15
SS pattern	16.65
S-β pattern	3.82
S-HPFH	1.41
Hb E trait	1.01
Hb D trait	0.9
Hb J	0.17
homozygous Hb E	0.12
homozygous Hb D	0.12
S-Hb D double heterozygous	0.1
S-Hb E double heterozygous	0.07
Hb Q India	0.02



Most common patterns were Sickle cell trait seen in 1172 cases (20.15%) and homozygous sickle cell anemia was seen in 969 cases (16.65%). This was common finding because central India is common belt for sickle cell anemia and trait. Diagnosis was made by correlating solubility test, Hb electrophoresis and HPLC findings which showed S window, along with considering percentage of Hb A and Hb F.

Hb S - β Thalassemia double heterozygous state was seen in 222 cases (3.82%), it showed S window and percentage of Hb A2 around 4-6%. The retention time for Hb A2 ranged between 3.59-3.67 minutes. On Hb electrophoresis SF A_2 Band was seen.

HbS - Hereditary persistence of fetal hemoglobin (HbS-HPFH) was seen in 82 cases (1.42%). Hb F percentage on HPLC was around 20-30% and on Hb electrophoresis SS pattern was seen.

Hb E trait was seen in 59 cases (1.01%). On HPLC Hb E presents as raised peak in the A2 region with retention times ranging from 3.68-3.79 minutes with Hb A_2 -> 7 %. Homozygous Hb E diagnosed in 7 (0.12%) cases with peak at A2 region on HPLC and high percentage of Hb E, ranging from 85-95% . Sickle cell - Hb E double heterozygous state diagnosed in 4 cases (0.07%) with A2 peak on HLPC and Hb E percentage around 30%. On Hb electrophoresis Hb E moves with Hb C and Hb O.

Hb D trait was seen in 52 cases (0.9%). On Hb electrophoresis AS pattern seen as HbD moves with Hb S and on HPLC D window was seen with retention time of 4.09- 4.14 min. Homozygous Hb D was seen in 7 (0.12%) cases with SS pattern was seen on Hb electrophoresis and D window on HPLC. Sickle cell - Hb D double heterozygous state diagnosed in 6 cases (0.1%) with SS pattern seen on Hb electrophoresis and D window was seen on HPLC.

Hb J diagnosed in 9 cases (0.17%). On Hb electrophoresis Hb J moves faster than Hb A. On HPLC - peak at P3 was seen with Retention time of 1.88 min. Values more than 15% -25% are indicating Hb J.

Hb Q diagnosed in only 1 case (0.02%). The characteristic findings include an unknown peak

(range 11-20%) on HPLC with a typical retention time of 4.77 plus/minus 0.01 min. On Hb electrophoresis Hb Q moves with Hb S.

Discussion

Sickle cell disease and hemoglobinopathies are prevalent in Central India region. Presentation of hemoglobinopathies can vary from, mild anemia to severe complications which can lead to disability and mortality.

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 use of cation-exchange high performance liquid chromatography (CE-HPLC) to separate and quantify various normal and abnormal Hb fractions has been increasing [3]. It offers a reliable tool for early, accurate detection; thereby aiding in prevention and management of various hemoglobinopathies [4].

Most common patterns were sickle cell trait and homozygous sickle cell anemia. This was common finding because Central India is common belt for sickle cell disease and hemoglobinopathies. Hb S homozygous presents as an S-Window with abnormal hemoglobin ranging from 70-90%. Values of Hb F generally are raised in parts of central India and Orissa [7]. 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. Diagnosis was made by correlating solubility test, Hb electrophoresis and HPLC findings which showed S window, along with considering percentage of Hb A and Hb F.

Presentation of Hb S - β Thalassemia double heterozygous state may be asymptomatic or may resemble Sickle cell disease. MCV is low, in range of 65- 75 fl, microcytes seen on peripheral blood smear. On HPLC S window is seen and percentage of Hb A2 around4-6%. The retention time for Hb A2 ranged between 3.59-3.67 minutes. Hb electrophoresis SF A_2 Band is seen

HbS - Hereditary persistence of fetal hemoglobin has extremely mild presentation and extremely good prognosis. All blood counts are generally normal. Hb F precentage on HPLC is around 20-30% and on Hb electrophoresis pattern SS pattern is seen.

Hb D Hemoglobinopathies- HbD moves with Hb S and on HPLC D window is seen with retention time of 4.09- 4.14 min.

Hb D trait- May have asymptomatic presentation or presents as mild anemia. All hematological parameters are generally normal. On Hb electrophoresis AS pattern is seen and D window seen on HPLC.

Homozygous Hb D- Can present with mild hemolytic anemia and mild to moderate splenomegaly. It occurs in first few months of life when Hb F levels fall. On Hb electrophoresis SS pattern seen and D window seen on HPLC.

Sickle cell - Hb D double heterozygous state- Can mimics sickle cell anemia clinically and hematologically. On Hb electrophoresis AS pattern seen and on HPLC D window is seen with retention time of 4.09-4.14 min [8, 10].

Hb E hemoglobinopathies

Hb E trait – Presentation may be asymptomatic or as mild anemia .Peripheral blood smear shows normocytic to microcytic RBCs, target cells are also seen. On HPLC Hb E presents as raised peak in the A2 region with retention times

ranging from 3.68-3.79 minutes with Hb A_2 -> 7 %.

Homozygous Hb-E - Presentation can be as mild anemia or completely asymptomatic. Peripheral blood smear shows microcytic RBCs and target cells. It is diagnosed by presence of peak at A2 region on HPLC and high percentage of Hb E, ranging from 85-95%.

Sickle cell - Hb E double heterozygous state – Presentation is usually asymptomatic or may presents as mild anemia. All blood counts are normal. It is diagnosed by presence of A2 peak on HLPC and Hb E percentage around 30%. On Hb electrophoresis Hb E moves with Hb C and Hb O [9, 10].

Hb J presents as elevated P3 peak on HPLC. Important is to note that a P3 peak up to six per cent is usually normal. Values six to 12% indicates sub optimal specimen. Values more than 15% -25% indicate Hb J [11]. Hb J is usually asymptomatic or patients may present with mild anemia. Hemoglobin electrophoresis at alkaline pH shows a fast moving band ahead of Hb A. The mutations can be seen either in alpha or beta chains. Hb J Meerut is an alpha chain variant with retention time of 1.88 minutes.

Hemoglobin Q-India is a rare alpha-chain structural variant caused by the mutation AAG-->GAG (Asp-->His) in the position of codon 64 of the alpha1 gene [12]. The characteristic findings include unknown peak (range 11-20%) on HPLC with a typical retention time of 4.77 plus/minus 0.01 min. On Hb electrophoresis Hb Q moves with Hb S.

Conclusion

- Various hemoglobinopathies have different clinical profile, thus their identification is important.
- Antenatal screening for hemoglobinopathies is important for preventing development of severe disease in new born, which can be achieved by counselling and prenatal diagnosis in select cases.
- Region wise documentation of prevalence of various hemoglobinopathies is essential for epidemiological studies.
- HPLC is best tool for screening of hemoglobinopathies and Thalassemia. However, DNA studies in select cases are essential for confirmation.

References

1. Kutlar F. Diagnostic approach to hemoglobinopathies. Hemoglobin, 2007; 31: 243-50.
2. WHO-executive board EB118/5, 118th Session Report by the Secretariat on Thalassaemia and other haemoglobinopathies: Prevalence of Haemoglobinopathies. May 2006, p. 1-8.
3. Higgins TN, Ridley B. Tentative identification of hemoglobin variants in the Bio - Rad variant II Hb A1C Method. Clin Biochem., 2005; 38: 272-7.
4. Riou J, Godart C, Hurtrel D, Mathis M, Bimet C, Bardakdjian-Michau J, et al. Cation-exchange HPLC evaluated for presumptive identification Of hemoglobin variants. Clin Chem., 1997; 43: 34-9.
5. Lt Col PK Gupta, Col H Kumar, Lt Col S Kumar, et al. Cation exchange high performance liquid chromatography for diagnosis of haemoglobinopathies. MJAFI, 2009; 65: 33-7.
6. Wild BJ, Bain BJ. Investigation of abnormal hemoglobins and thalassemia. In: Bain BJ, Bates I, Laffan MA, Lewis SM, editors. Dacie and Lewis Practical Hematology. 11th ed., China: Elsevier; 2011, p. 301-32.
7. Kar BC, Devi S. Clinical profile of sickle cell disease in Orissa. Indian J Pediatr., 1997; 64: 73-7.
8. Zeng YT, Huang SZ, Zhou LD, Huang HJ, Jiao CT, Tang ZG, et al. Identification of hemoglobin D Punjab

by gene mapping. Hemoglobin, 1986; 10: 87-90.

9. Kishore B, Khare P, Gupta RJ, Bisht S, Majumdar K. Hemoglobin E disease in North Indian population: A report of 11 cases. Hematology, 2007; 12: 343-7.
10. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indianpopulation using HPLC: Report of 2600 cases. Indian J Pathol Microbiol [serial online] 2010 [cited 2017 Dec 20]; 53: 57-62.
11. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: A study of 60000 samples in a clinical diagnostic laboratory. Clin Chem., 2004; 50: 1736-47.
12. Desai DV, Dhanani H, Kapoor AK, Yeluri SV. HbQ-India in a Sindhi family: An uncommon hemoglobin variant. Lab Hematol., 2004; 10: 212-4.