ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 03 | March - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SPECTRUM OF COMPLEX HEMOGLOBINOPATHIES DIAGNOSED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY: A CASE SERIES

Haematology

Dr. Vaishnavi Jaiswal Senior Resident, Department of Pathology, Indira Gandhi Government Medical College, Nagpur.

ABSTRACT

Background: Hemoglobinopathies constitute a major group of inherited hematological disorders, particularly prevalent in the Indian subcontinent. Accurate identification of these disorders is essential for appropriate clinical management and genetic counseling. High-performance liquid chromatography (HPLC) has emerged as a reliable and widely used technique for the detection and characterization of hemoglobin variants. **Aim:** To evaluate the utility of HPLC in identifying compound and double heterozygous hemoglobinopathies and to highlight characteristic chromatographic patterns encountered in routine laboratory practice. **Materials And Methods:** This descriptive case series included five patients evaluated for suspected hemoglobinopathies. Venous blood samples collected in EDTA were analyzed using cation-exchange high-performance liquid chromatography. Hemoglobin fractions including HbA, HbA□, HbF, and variant hemoglobins were quantified and interpreted in correlation with complete blood count parameters, peripheral blood smear findings, and available clinical details. **Results:** HPLC analysis revealed a spectrum of hemoglobinopathies comprising compound heterozygous HbS–HPFH, sickle β-thalassemia heterozygous state, double heterozygous HbS–HbE, double heterozygous HbD–HbS, and HbAE. Distinct chromatographic patterns enabled accurate identification of these hemoglobin variants. Correlation with hematological parameters supported the HPLC-based diagnosis in all cases. **Conclusion:** HPLC is a reliable and effective diagnostic modality for the evaluation of hemoglobinopathies, including complex compound and double heterozygous states. Careful interpretation of chromatographic patterns, along with hematological and clinical correlation, enhances diagnostic accuracy. Despite the small sample size, this case series underscores the practical utility of HPLC in routine diagnostic settings.

KEYWORDS

Hemoglobinopathies; High-performance liquid chromatography; HbS; HbE; HbD; Thalassemia

INTRODUCTION

Hemoglobinopathies comprise a diverse group of inherited disorders resulting from qualitative or quantitative abnormalities of hemoglobin synthesis.. Conditions such as thalassemia syndromes and structural hemoglobin variants contribute significantly to morbidity and impose a considerable socioeconomic burden, highlighting the importance of early and accurate diagnosis for optimal clinical management and genetic counseling.[1,2]

High-performance liquid chromatography (HPLC) has emerged as a reliable and widely accepted method for the detection and characterization of hemoglobin variants. The technique separates hemoglobin fractions based on differences in ionic interactions and retention time, allowing precise identification and quantification of HbA, HbA□, HbF, and abnormal hemoglobins.[3]

HPLC offers superior resolution, higher sensitivity, excellent reproducibility, and rapid turnaround time, making it suitable for routine laboratory use.□,□

Distinct chromatographic patterns, when interpreted in conjunction with red cell indices and peripheral smear findings, can strongly suggest specific hemoglobinopathies. However, certain limitations persist, including overlapping retention times, influence of iron deficiency, recent blood transfusions, and coexistence of multiple hemoglobin variants, which may complicate interpretation in some cases.□,□

Such reports aid in reinforcing pattern recognition and enhancing diagnostic confidence among laboratory physicians and pathologists.□

The present case series describes the HPLC findings in five patients evaluated for suspected hemoglobinopathies, with emphasis on the spectrum of chromatographic patterns observed and the importance of correlating HPLC results with hematological and clinical findings.

AIM

To study and analyze the high-performance liquid chromatography (HPLC) patterns in selected cases of hemoglobinopathies and to highlight the diagnostic utility of HPLC in identifying compound and double heterozygous hemoglobin disorders.

MATERIALS AND METHODS

This descriptive case series was conducted in the hematology laboratory of a tertiary care center. A total of five cases referred for evaluation of suspected hemoglobinopathies were included in the study. The cases were selected based on distinct and representative high-performance liquid chromatography (HPLC) patterns, particularly compound and double heterozygous hemoglobin disorders.

Venous blood samples were collected in EDTA anticoagulated vacutainers and processed within the recommended time frame. Hemoglobin analysis was performed using high-performance liquid chromatography (HPLC) on an automated hemoglobin analyzer. The method is based on cation-exchange chromatography, which separates hemoglobin fractions according to their ionic interactions and retention times.□,[1]□

The hemoglobin fractions analyzed included HbA, HbA□, HbF, and abnormal hemoglobin variants such as HbS, HbE, and HbD. Chromatographic patterns were interpreted using manufacturer-provided reference ranges and established diagnostic criteria described in standard hematology literature.[11]

Complete blood count (CBC) parameters and peripheral blood smear examination were reviewed wherever available to support the interpretation of HPLC findings. The final diagnosis in each case was established by correlating HPLC patterns with hematological parameters and clinical details.

RESULTS

A total of five cases evaluated for suspected hemoglobinopathies were included in the present case series. All patients underwent hemoglobin analysis by high-performance liquid chromatography (HPLC). The chromatographic patterns were interpreted in conjunction with clinical details, complete blood count parameters, and peripheral smear findings.

Case 1 showed a chromatographic pattern consistent with a compound heterozygous state for HbS and hereditary persistence of fetal hemoglobin (HPFH), characterized by a significant HbF fraction along with the presence of HbS and reduced or absent HbA.

Case 2 demonstrated features suggestive of sickle beta-thalassemia heterozygous state, with elevated HbS, increased HbA□ levels, variable HbF, and reduced HbA fraction.

Case 3 revealed a double heterozygous state for HbS and HbE, showing distinct peaks corresponding to HbS and HbE, along with reduced HbA.

Case 4 exhibited a double heterozygous state for HbD and HbS, characterized by the presence of HbS and an abnormal hemoglobin

fraction corresponding to HbD, with minimal or absent HbA.
Case 5 showed a chromatographic pattern consistent with HbAE, with a predominant HbA fraction and a significant HbE peak.

The HPLC patterns in all five cases were characteristic and allowed accurate classification of the hemoglobinopathies when correlated with hematological parameters.

Table 1: HPLC Findings And Final Diagnosis In The Case Series

Case No.	Hemoglobin Pattern on HPLC	Key HPLC Features	Final Diagnosis
1	HbS + increased HbF	Prominent HbF peak, presence of HbS, reduced/ absent HbA	Compound heterozygous HbS–HPFH
2	HbS + raised HbA□ ± HbF	Elevated HbS, increased HbA□, reduced HbA	Sickle β-thalassemia (heterozygous)
3	HbS + HbE	Distinct HbS and HbE peaks, reduced HbA	Double heterozygous HbS–HbE
4	HbS + HbD	Presence of HbS and HbD peaks, minimal/absent HbA	Double heterozygous HbD–HbS
5	HbA + HbE	Predominant HbA with significant HbE peak	

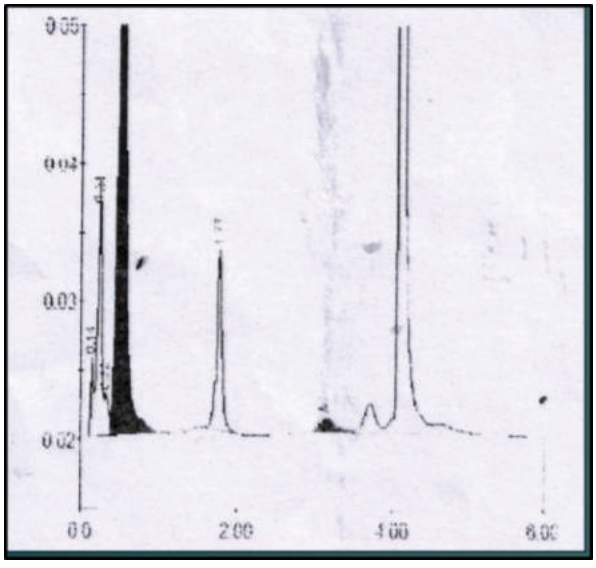

Figure 1 History : A 7 years old female child came with complaints of fever since last 3 days, pain in both knees and left elbow since last 2 days.

HPLC findings were suggestive of compound heterozygous for HB S and Heriditary Persistence of Fetal hemoglobin.

Findings were Hb - 7.40gm/dl, HCT – 21.80, RBC – 2.44, RDW – 16.80, HbF – 23.9, HbS - 65.5, MCV – 89.30, MCHC – 34.0, MCH – 30.40

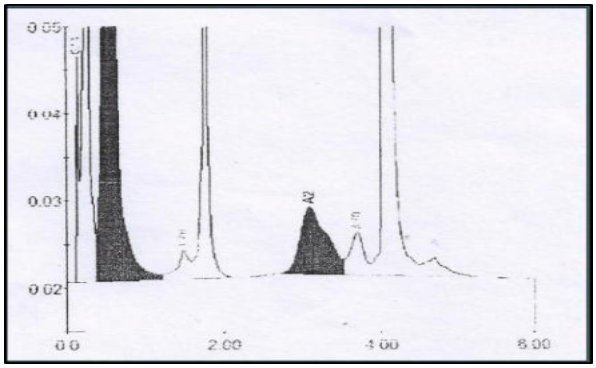

Figure 2. A 4 years old male child came with complaints of fever, cough, cold since last
7 days. HPLC findings were suggestive of sickle beta thalassemia heterozygous.
Findings were Hb – 5.80, HCT – 17.80, RBC – 2.73, RDW – 20.80, HbS – 53.6, HbA2 – 5.0
HbF – 27.0, MCV – 65.2, MCHC -32.50, MCH – 21.20

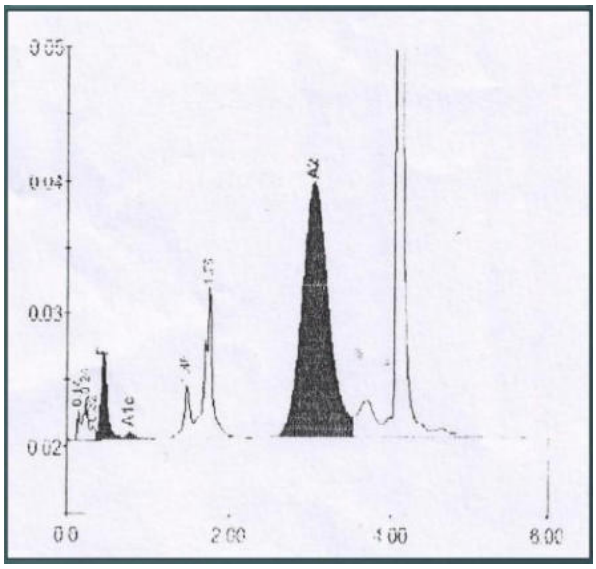

Figure3- A 3 years old male child came with complaints of enteric fever associated with chills, cough, cold since last 7 days.

Hb – 9.1, HCT – 25.10, RBC – 3.87, RDW – 14.9, HbS – 57.1, HbA2 – 39.0, MCV – 64.9 MCHC – 36.3, MCH -23.6.

HPLC findings were suggestive of double heterozygous for HbS and HbE

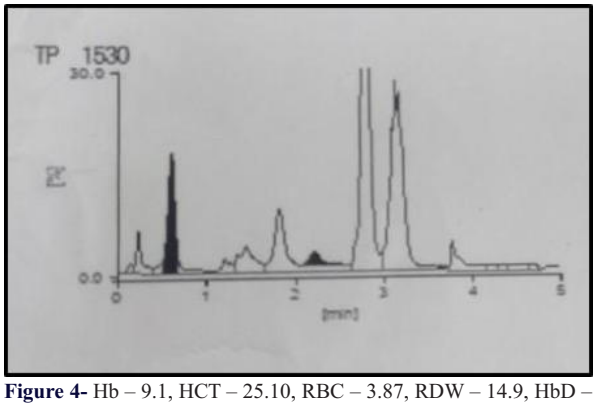

Figure 4- Hb – 9.1, HCT – 25.10, RBC – 3.87, RDW – 14.9, HbD – 38.1, HbS - 26.2 HbA2 – 3.2, HbF- 13.2, MCV – 64.9 MCHC – 36.3, MCH -23.6.

HPLC findings were suggestive of double heterozygous for HbD and HbS

A 14 years old male child came with complaints of fever, cough, cold since last 4 day

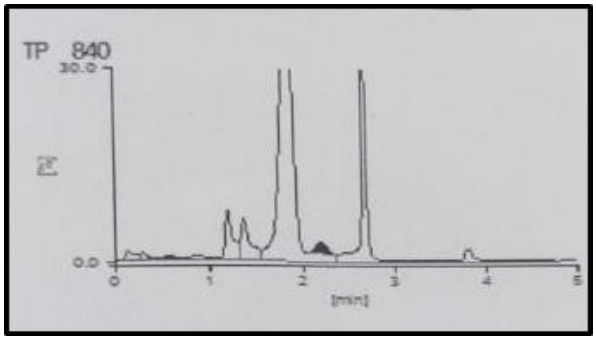

Figure 5 Hb – 9.1, HCT – 25.10, RBC – 3.87, RDW – 14.9, HbE – 20.3, HbA2 – 3.1, HbF- 1.6, MCV – 64.9 MCHC – 36.3, MCH -23.6. HPLC findings were suggestive of AE
A 15 years old male child came with complaints of fever, cough, cold since last 4 day

DISCUSSION

High-performance liquid chromatography (HPLC) is widely recognized as a reliable and reproducible method for the detection and characterization of hemoglobinopathies. Owing to its ability to separate and quantify hemoglobin fractions with high precision, HPLC has become a frontline diagnostic tool in both screening and confirmatory settings. Several large studies have demonstrated its superiority over conventional electrophoretic techniques, particularly in identifying complex and compound heterozygous hemoglobin disorders.[12,13]

In the present case series, HPLC successfully identified a spectrum of hemoglobinopathies, including compound and double heterozygous states. Similar findings have been reported by Rao et al. and Colah et al., who documented that characteristic alterations in HbA, HbA□, HbF, and variant hemoglobins form the basis of diagnosis using cation-exchange HPLC.[1□,1□] Elevated HbF levels, commonly encountered in certain compound hemoglobinopathies, have been shown to influence both chromatographic patterns and disease severity, particularly in disorders involving HbS.[1□,1□]

The role of HPLC in differentiating sickle cell–related disorders has been extensively studied. Previous authors have emphasized that quantitative assessment of HbA□ is critical in distinguishing sickle cell trait from sickle β-thalassemia, as raised HbA□ levels are a consistent feature of the latter.[1□,1□] These observations are concordant with findings in the present series, reinforcing the diagnostic value of HPLC in routine practice.

Studies have also highlighted the importance of HPLC in detecting combinations involving HbE and HbD. Joutovsky et al. demonstrated that retention time analysis is a powerful diagnostic tool, although careful interpretation is required due to overlapping chromatographic windows in certain variants.[13] Indian studies have further reported that compound heterozygous states involving HbS, HbE, and HbD may present with variable hematological and clinical profiles, underscoring the importance of accurate laboratory diagnosis.[1□,1□]

Despite its advantages, HPLC has certain limitations. Factors such as iron deficiency anemia, recent blood transfusions, and coexistence of multiple hemoglobin variants can influence hemoglobin fractions and complicate interpretation.[2□] Therefore, most authors recommend that HPLC findings be interpreted in conjunction with complete blood count parameters, peripheral smear examination, and clinical details. Molecular studies and family screening are advised in selected cases for definitive confirmation.

Although limited by a small sample size, the present case series contributes to existing literature by documenting diagnostically challenging hemoglobinopathies and reinforcing the practical utility of HPLC in routine laboratory settings. Similar small case series and observational studies have played an important role in improving recognition and interpretation of uncommon hemoglobin variant patterns.[1□,1□]

CONCLUSION

High-performance liquid chromatography (HPLC) is a robust, reproducible, and sensitive technique for the evaluation of hemoglobinopathies and remains a cornerstone in routine diagnostic hematology. The present case series highlights the effectiveness of HPLC in identifying not only common hemoglobin variants but also compound and double heterozygous hemoglobinopathies, which often present diagnostic challenges. Several studies have similarly emphasized that characteristic chromatographic patterns, when combined with quantitative estimation of HbA, HbA□, and HbF, allow accurate classification of hemoglobin disorders.[21,22]

The findings of this study further reinforce that interpretation of HPLC results should always be integrated with complete blood count parameters, peripheral smear examination, and clinical correlation to ensure diagnostic accuracy. Despite advances in molecular diagnostics, HPLC continues to serve as a practical and reliable first-line investigative modality, particularly in resource-limited settings.[23]

REFERENCES

1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704–712.
2. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480–487.
3. Bain BJ. Haemoglobinopathy diagnosis. 2nd ed. Oxford: Blackwell Publishing; 2006.
4. Wild BJ, Bain BJ. Investigation of abnormal hemoglobins and thalassemia. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis Practical Haematology. 11th ed. Philadelphia: Elsevier Churchill Livingstone; 2012. p. 231–268.
5. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60000 samples. Am J Clin Pathol. 2004;122(3):442–450.
6. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of hemoglobinopathies diagnosed by cation exchange HPLC and modulating effects of nutritional deficiency anaemias from north India. Indian J Med Res. 2010;132:513–519.
7. Sharma P, Das R, Trehan A, Bansal D, Chhabra S, Kaur J, et al. Hemoglobinopathies in India: a large population-based study. Indian J Pathol Microbiol. 2013;56(3):210–215.
8. Colah R, Gorakshakar A, Nadkarni A. Global burden, distribution and prevention of beta-thalassemias and hemoglobin E disorders. Expert Rev Hematol. 2010;3(1):103–117.
9. Bain BJ. Haemoglobinopathy diagnosis. 2nd ed. Oxford: Blackwell Publishing; 2006.
10. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60000 samples. Am J Clin Pathol. 2004;122(3):442–450.
11. Wild BJ, Bain BJ. Investigation of abnormal hemoglobins and thalassemia. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis Practical Haematology. 11th ed. Philadelphia: Elsevier Churchill Livingstone; 2012. p. 231–268.
12. Wild BJ, Bain BJ. Investigation of abnormal hemoglobins and thalassemia. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis Practical Haematology. 11th ed. Philadelphia: Elsevier; 2012. p. 231–268.
13. Joutovsky A, Hadzi-Nesic J, Nardi MA. HPLC retention time as a diagnostic tool for hemoglobin variants and hemoglobinopathies: a study of 60,000 samples. Am J Clin Pathol. 2004;122(3):442–450.
14. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of hemoglobinopathies diagnosed by cation exchange HPLC and modulating effects of nutritional deficiency anaemias from North India. Indian J Med Res. 2010;132:513–519.
15. Colah R, Surve R, Wadia M, Solanki P, Mayekar P, Thomas M, et al. HPLC studies in hemoglobinopathies. Indian J Hum Genet. 2007;13(2):71–76.
16. Steinberg MH. Fetal hemoglobin in sickle cell anemia. Blood. 2009;114(12):2309–2318.
17. Thein SL. Genetic modifiers of sickle cell disease. Haematologica. 2005;90(5):649–660.
18. Sharma P, Das R, Trehan A, Bansal D, Chhabra S, Kaur J, et al. Hemoglobinopathies in India: a large population-based study. Indian J Pathol Microbiol. 2013;56(3):210–215.
19. Jain DL, Sarathi V, Desai S, Bhatnagar M. HbD-Punjab: clinical and hematological profile. IndianJ Pediatr. 2012;79(5):650–654.
20. Bain BJ. Diagnosis from the blood smear. N Engl J Med. 2005;353(5):498–507.
21. Bain BJ. Haemoglobinopathy diagnosis. 2nd ed. Oxford: Blackwell Publishing; 2006.
22. Colah R, Gorakshakar A, Nadkarni A. Global burden, distribution and prevention of beta-thalassemias and hemoglobin E disorders. Expert Rev Hematol. 2010;3(1):103–117.
23. Wild BJ, Bain BJ. Investigation of abnormal hemoglobins and thalassemia. In: Lewis SM, Bain BJ, Bates I, editors. Dacie and Lewis Practical Haematology. 11th ed. Philadelphia: Elsevier; 2012. p. 231–268.