CLINICAL STUDY

# Uncovering Silent Hemoglobinopathies: Utility of HPLC in Outpatient Screening

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## ABSTRACT

**Background:** Hemoglobinopathies are among the most common inherited red cell disorders globally and pose significant public health burden in India. Differentiating hemoglobinopathies from nutritional anemia, particularly in antenatal women, remains a diagnostic challenge. The aim was to determine prevalence and spectrum of undiagnosed hemoglobinopathies among patients attending outpatient clinics using high-performance liquid chromatography (HPLC). **Methods:** A cross-sectional observational study was conducted from January 2022 to December 2024. A total of 7,440 blood samples from patients attending general medicine, pediatrics, and antenatal OPDs were analyzed. Patients with a known diagnosis of hemoglobinopathy, recent blood transfusion (within 1 year) and patients with uncontrolled diabetes were excluded. Complete blood count (CBC), peripheral smear, sickling tests, and HPLC chromatograms were analyzed. **Results:** A total of 7,440 outpatient samples were analyzed and a female preponderance (81.2%) was seen in the study population. Among these, 162 cases (2.17%) were found to have abnormal hemoglobin (Hb) fractions suggestive of hemoglobinopathy on HPLC. The most common hemoglobinopathy identified was the heterozygous beta-thalassemia trait (1.37%), followed by heterozygous HbAE (0.32%), heterozygous HbAS or sickle cell trait (0.18%), and heterozygous HbAD Punjab (0.14%). Rare Hb variants included homozygous sickle cell disease (HbSS) (0.04%), hereditary persistence of fetal hemoglobin (HPFH) (0.05%), and 1 case each (0.01%) of homozygous HbEE, heterozygous HbAC, compound HbE/beta-thalassemia, and delta–beta thalassemia. Additionally, 5 cases (0.06%) showed unidentified abnormal peaks on HPLC that required further molecular testing. Peaks in the P03 and P04 retention windows were correlated with elevated HbA1c in individuals with diabetes and excluded from hemoglobinopathy classification. **Conclusion:** HPLC is a reliable, rapid, and sensitive tool for detecting both common and rare hemoglobinopathies in outpatient settings. Its integration into routine antenatal and general screening can facilitate early diagnosis, genetic counseling, and preventive interventions. Expanding access to HPLC testing can reduce the burden of transfusion-dependent hemoglobinopathies and improve long-term patient outcomes.

**Keywords:** Hemoglobinopathies, beta-thalassemia trait, HPLC, carrier detection, sickle cell trait, antenatal screening, genetic counseling

Hemoglobinopathies are among the most common inherited monogenic red cell disorders worldwide, which affect structure or production of hemoglobin (Hb). They generally result from genetic mutations in the globin genes, which can include insertions, deletions, or substitutions of amino acids in either the $\alpha$-globin or non-$\alpha$-globin genes[1].

Approximately 7% of the worldwide population carries a clinically significant hemoglobinopathy, with more than 270 million carriers globally. Nearly 3 to 5 lakhs newborn with serious form of hemoglobinopathies are born every year, of which nearly 90% are born in middle- or low-income countries[2]. Hemoglobinopathies contribute to nearly 3.4% of mortality in children up to 5 years of age worldwide[3]. Estimates indicate that across all age groups, there are approximately 24.7 million carriers of beta-thalassemia and 16.9 million carriers of sickle cell anemia in India[4].

India accounts for 10% of the global thalassemia burden, with approximately 10,000 children born annually with thalassemia major. Hemoglobinopathy is identified in approximately 7% of pregnancies and nearly 1% of couples are at risk for this condition[3].

The clinical presentations of various hemoglobinopathies can range from asymptomatic to severe, transfusion-

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dependent anemia, accompanied by other fatal complications. Therefore, early detection and accurate diagnosis of these disorders are crucial to prevent the occurrence of transfusion dependent hemoglobinopathies and reduce the associated economic and psychological burdens of a lifelong condition[5].

In India, the country faces a dual burden of nutritional anemia and hemoglobinopathies, which often leads to delayed or missed diagnosis. Pregnant women who are carriers of hemoglobinopathies often present with mild to moderate anemia, similar to those with nutritional anemia. Diagnosing hemoglobinopathy is crucial, as it enables parents to make informed reproductive decisions and alerts health care providers to the carrier status. Estimating the prevalence of beta-thalassemia carriers in India has been hindered due to challenges faced in implementing systematic sampling methods and adopting international diagnostic criteria and laboratory standards[4].

The objective of the current study was to assess the prevalence of undiagnosed hemoglobinopathy among a general population attending OPD clinics by high-performance liquid chromatography (HPLC). It is a rapid, sensitive, and reproducible method used in the tertiary care centers, aimed at facilitating early intervention strategies for the identification, diagnosis, and prevention of severe hemoglobinopathies in future generations.

## MATERIALS AND METHODS

The present study has been conducted over a period of 2 years, from January 2022 to December 2024, using samples analyzed by HPLC at a tertiary care center in New Delhi. A total of 7,440 samples were studied, of which 162 (2.17%) exhibited an abnormal Hb fraction, while 7,278 (97.8%) had a normal Hb fraction according to HPLC analysis.

This is a cross-sectional observational study that included HPLC analysis of patients attending the general medicine, pediatrics and antenatal OPD clinics for routine check-ups and who were advised for HPLC analysis for any refractory anemia, known family history regardless of their age, from 1.5 to 50 years.

Patients with previously diagnosed hemoglobinopathy and with recent blood transfusion history less than 1 year were excluded from our study. Majority of our study subjects were female (81.2%), as we included individuals attending antenatal OPD for routine checkups and this population represented the general population albeit from lower socioeconomic group. Consent was taken from all study participants and ethical clearance from our Institutional Ethical Committee.

Whole blood samples (3 mL) were collected from all the patients selected for the study in the OPD clinic using K2EDTA vials. A complete blood count (CBC) with a peripheral blood smear, along with HPLC testing, was performed within 24 hours. The quantitative percentage of Hb components was measured using Tosoh automated Hb analyzer HLC-723G11 (Fig. 1), in variant analysis mode (Beta-thalassemia mode). This system operates on the principle of HPLC with a cation-exchange column. The system separates Hb components into six fractions within a 1-minute time span. No sample pre-treatment was required.

HPLC is a form of column chromatography. It involves passing a mixture, which contains the analyte, through a column (stationary phase), with a liquid (mobile phase) at high pressure. Cation exchange chromatography is a process that allows the separation of the mixture based on the charge properties of the molecules in the mixture. Cation exchange chromatography retains analyte molecules based on columbic (ionic) interactions. The stationary phase surface displays negatively charged functional groups that interact with positively charged cations in the mixture. The separated Hbs then pass through the flow cell of the filter photometer, where changes in the absorbance at 415 nm are measured. Hemoglobin A2/hemoglobin F (HbA2/F) calibrator and two levels of controls were analyzed at the beginning of each run. Results are shown on the very straightforward high-resolution chromatograms.

A sickling test using freshly prepared 1% sodium metabisulfite solution was performed for all the heterozygous or homozygous hemoglobin S (HbS) cases. The sodium metabisulfite quickly reduces oxygen, causing sickling of red blood cells.

**Figure 1.** HLC-273G11 analyzer for HPLC.

## RESULTS

Various types of the Hb abnormalities were detected using HLC-723G11 analyzer based on their retention time and area percentage. Checkpoints of chromatogram that are considered include flat baseline, peak shape, presence of three defined peaks HbF (fetal hemoglobin), HbA0 (adult hemoglobin A0), and HbA2 (adult hemoglobin A2), variant windows of HbE (hemoglobin E), HbD (hemoglobin D), HbS, and HbC (hemoglobin C), absence of undefined unknown peaks, retention time, area, and presence of any flag (A flag in a hemochromatogram means the machine has found something unusual or abnormal in the blood sample). The total acceptable area was 900-5,000. Values outside this range were not accepted and such samples were manually prediluted and rerun. The retention time with windows assigned to various Hb fractions provided by the manufacturer is shown in Table 1.

Out of the total 7,440 samples analyzed, 162 (2.17%) exhibited hemoglobinopathy, while 7,278 (97.8%) showed normal Hb fraction according to HPLC analysis. The most common hemoglobinopathy observed was heterozygous beta-thalassemia, with 101 (1.37%) cases, followed by heterozygous HbAE in 23 (0.32%) cases, heterozygous HbAS (sickle cell trait) in 12 (0.18%) cases and heterozygous HbAD Punjab in 10 (0.14%) cases.

Less common abnormal Hbs identified included homozygous sickle cell disease in 3 (0.04%) cases, hereditary persistence of fetal hemoglobin (HPFH) in 4 (0.05%) cases, and 1 (0.01%) each case of homozygous HbEE, heterozygous HbAC, compound heterozygous HbE-beta thalassemia and Delta-beta thalassemia. There were also 5 (0.06%) cases with abnormal unidentified peaks on HPLC (Table 2).

The primary abnormality identified in our study was heterozygous beta-thalassemia, diagnosis based on the HbA2 value and further correlated with CBC and peripheral smear examination. The retention time for HbA2 typically ranges between 2.08 minutes and 2.30 minutes, with a cut-off value of 3.5%. Majority hold >4.5% HbA2 level. On all HbS-positive cases, sickling test was performed for ensuring that other Hb variants with similar retention times, which might elute alongside HbS, were excluded. For cases of HPFH, family members were screened by HPLC for HPFH; however, they were not included in the study population. For those with abnormal or unidentified results, further molecular analysis was recommended for categorization. Significant abnormal peaks, identified with a cut-off value of >10% and falling within P03 and P04, with a mean retention time of 1.2 minutes, were further investigated for any history of diabetes, and HbA1c testing was performed and those having high HbA1c, were also excluded from the study population.

**Table 1.** Interpretation of Various Hb Fractions Based on their Retention Times in HPLC

| Hb Fractions | Retention Time (min) |
|---|---|
| HbF | 0.5-0.7 |
| HbA0 | 1.7-2.0 |
| HbA2 | 2.05-2.35 |
| E+ | 2.65-2.73 |
| D+ | 2.74-2.90 |
| S+ | 2.99-3.40 |
| C+ | 3.70-5.0 |

Very high HbF > 50% may shift peak after 0 .70 minutes as PO2/PO3 Peak.

**Table 2.** Distribution of Hemoglobin Patterns in the Study Population

| Hemoglobin pattern | Number of cases | Percentage (%) |
|---|---|---|
| Normal Hb fraction | 7,278 | 97.8 |
| Heterozygous beta-thalassemia | 101 | 1.37 |
| Heterozygous HbAE | 23 | 0.32 |
| Heterozygous HbAS (Sickle cell trait) | 12 | 0.18 |
| Heterozygous HbAD (HbD Punjab trait) | 10 | 0.14 |
| HPFH | 04 | 0.05 |
| Homozygous HbSS (Sickle cell disease) | 03 | 0.04 |
| Heterozygous HbAC | 01 | 0.01 |
| Homozygous HBEE | 01 | 0.01 |
| Compound HbE-beta-thalassemia trait | 01 | 0.01 |
| Delta-beta-thalassemia | 01 | 0.01 |
| Abnormal peak | 05 | 0.06 |
| **Total** | **7,440** | **100** |

## DISCUSSION

Hemoglobinopathies, including thalassemia and sickle cell disease, are major health concerns in India, with their prevalence varying across different populations due to the country's diverse ethnic and genetic

backgrounds, particularly in tribal populations in states like Maharashtra, Madhya Pradesh, Chhattisgarh, Odisha, and Gujarat[6,7]. Thalassemia, particularly beta-thalassemia is prevalent, with an estimated 3%-4% of the Indian population carrying the trait[8]. These disorders represent a major public health challenge in India, which has prompted increased screening and prevention efforts, such as newborn screening programs and carrier testing.

Understanding the prevalence of lifelong genetic disorders is crucial for effective health care management, as well as for guiding prenatal genetic counseling. By conducting comprehensive studies on these conditions, health care professionals can gain valuable insights into their distribution and frequency, which in turn aids in early detection and timely medical intervention. Early diagnosis through genetic screening and counseling empowers individuals and families to make informed decisions, potentially reducing the long-term impact of these disorders.

This study has been conducted in Delhi, the national capital, which holds unique significance due to its diverse and dynamic population. As a major metropolitan center, Delhi attracts a large number of migrants from across the country. This diversity allows the findings of the study to not only reflect the central region but also provide insights applicable to various other regions of India. Consequently, the data derived from this research can contribute meaningfully to national strategies for genetic disorder awareness, prevention, and management.[9]

Hemoglobinopathy is confirmed by HPLC as the gold standard[10] at various centers of India instead of conventional methods including Hb electrophoresis (gel electrophoresis), capillary electrophoresis and HbF estimation by alkali denaturation for thalessemia, which are less precise, time-consuming. Many a times, these conventional methods are unable to detect other Hb variants with same electrophoretic mobility and various Hb fraction in single step process for identification of Hb variants. Other methods for Hb fractions estimations are liquid chromatography-mass spectrometry and DNA-based molecular genetic testing. Both techniques are extremely precise and highly accurate; but they require high-end equipment, which are very expensive as well as highly skilled operators, making them unsuitable for routine testing. So, cation exchange HPLC remains the gold standard for confirming the diagnosis of hemoglobinopathies, which accurately identifies most of the commonly found Hb variants, except for a few rare variant hemoglobins with same retention time, which require molecular analysis for confirmation.

In the present study, cation-exchange HPLC was performed by Tosoh automated Hb analyzer HLC-723G11. The prevalence of various hemoglobinopathies was lower in our study (2.21%) compared to the studies by Singh et al 2024 (20.12%)[2] in western India and Mondal et al 2016 (12.17%)[10] in eastern India. This could be attributed by factors such as difference in ethnic composition and geographic location of the population as hemoglobinopathies are more common in specific groups. Additionally, variation in sample size, health care access and public health interventions could lead to discrepancies in detection rates and differences in prevalence.

Our study had a female preponderance (81.2% vs.18.7%), which correlates well with the studies done by Singh et al 2024[2] and Pathak et al 2024[11], since they were advised for HPLC screening as part of routine antenatal checkup.

Heterozygous beta-thalassemia was the major abnormality detected (1.35%), which was also the most common hemoglobinopathy detected by Singh et al 2024 (15.75%)[2] and by Mondal et al 2016 (4.60%)[10].

The borderline HbA2 levels between 3.5 and 4.0 should be carefully investigated along with CBC and peripheral smear examination. Sahoo et al 2023[12] showed that megaloblastic anemia can also lead to a false positive diagnosis of heterozygous beta-thalassemia. Repeat HPLC should be done after adequate supplementation of vitamin B12 and folic acid.

Heterozygous HbAE was the second most common hemoglobinopathy detected in our study with occurrence of 0.3%. This was lower than the prevalence reported by Singh et al 2024[2] (1.44%) and Mondal et al 2016[10] (3.02%).

However, the overall pattern of hemoglobinopathy distribution in our study aligns with that observed by Mondal et al 2016[10] in eastern India, where most common condition was beta-thalassemia trait followed by HbE trait. In their study, beta-thalassemia trait and HbE trait were reported as 4.6% and 3.02%, respectively, compared to 1.35% and 0.3% in our findings.

Patients with HbE trait are asymptomatic, but those with compound HbE beta-thalassemia present with a variable clinical picture ranging from a condition indistinguishable from beta-thalassemia major requiring blood transfusions during infancy to a mild form of thalassemia intermedia showing mild asymptomatic anemia. It is said that worldwide approximately 50% of severe beta-thalassemia major patients are of HbE beta-thalassemia[13]. In our study, 1 case (0.01%) was detected

as compound HbE beta-thalassemia, which is lower than study done by Mondal et al 2016[10].

Interestingly, in a study done in Odisha, sickle-cell trait was the most common abnormality found[14]. In the present study, sickle-cell trait was found in 0.16% as the third most common corresponding to similar prevalence in the study done by Mondal et al 2016 (0.38%) in eastern India[10].

Heterozygous HbAD Punjab is another beta chain variant with relatively low frequency and highest prevalence in Punjab region 2%-3%, 1% in Gujarat[15]. We found 10 (0.13%) cases of HbAD Punjab, which was lower compared to those reported by Mohanty et al[16] with 1.09% in Ludhiana and 0.48% by Singh et al[2]. Other abnormal hemoglobins identified included homozygous sickle cell disease in 3 (0.04%) cases, HPFH in 4 (0.05%) cases, and 1 each case (0.01%) of homozygous HbEE, heterozygous HbAC, and delta–beta thalassemia.

Delta–beta thalassemia is a rare cause of elevated fetal Hb. Very few cases have been reported from India[17]. HPFH has also been reported infrequently from different regions of India[18]. In this study, the difference between the two was based on the percentage of HbF, the mean corpuscular volume, and the red blood cell distribution width. In the ICMR multicenter study, the incidence of delta–beta thalassemia was 0.73% and that of HPFH was 0.18%, which in our study was 0.01% and 0.04%, respectively[19].

Out of 5 (0.06%) cases with abnormal unidentified peaks on HPLC, 3 cases showed an abnormal peak at P05 window with retention times ranging from 1.25 to 1.87 minutes, and 1 case each at P06 window with retention time of 1.91minutes and at P08 window with retention time of 2.56 minutes. These cases were further advised for molecular testing but were lost to follow-up. Additionally, 6 cases fell into P03 and P04 window with retention time of 1.2 minutes. After confirming history of diabetes, their HbA1c levels were ranging from 7.5% to 11.5%. These cases were excluded from our study. Based on our findings, we recommend that patient with abnormal peak at P03 and P04 window should carefully be investigated for diabetes rather than advising molecular studies.

HPLC is a valuable diagnostic tool for identifying and quantifying Hb fractions, due to its sensitivity, precision, and rapid processing capabilities. However, it has certain limitations. For example, in some cases of beta-thalassemia, particularly in coexisting iron deficiency, HbA2 levels may remain within normal range, potentially leading to misdiagnosis. Additionally, some Hb variants may co-elute making it difficult to distinguish solely by HPLC. When interpreting chromatograms, it is essential to consider the potential influence of nutritional anemias. For instance, iron deficiency can lower HbA2 levels, potentially masking the beta-thalassemia trait, while cobalamin (vitamin B12) or folate deficiency may elevate HbA2 levels, leading to a false diagnosis of thalassemia trait[12,20-22]. Therefore, when necessary, HPLC results should be followed by molecular testing, such as polymerase chain reaction, amplification refractory mutation system, or other similar methods, to accurately identify specific mutations responsible for Hb disorders.

## CONCLUSION

In conclusion, HPLC is an accurate, rapid, sensitive, and reproducible method for early detection and quantification of most hemoglobinopathies and their variants. It serves as an ideal alternative to traditional techniques in routine clinical laboratories with heavy workloads, offering an efficient way to screen for various hemoglobinopathies in conjunction with CBC and iron studies. Premarital and antenatal screening serve as vital strategies in reducing the incidence of beta-thalassemia major in newborns. These screening programs should be supported through collaboration among central and state governments, nongovernmental organizations, and health care institutions.

Expanding access to HPLC testing is crucial for improving the early diagnosis and management of thalassemia major and other hemoglobinopathies. Early and accurate detection allows for timely medical intervention, genetic counseling, and appropriate monitoring, which can prevent complications and reduce the number of patients who progress to transfusion-dependent thalassemia. This not only improves the quality of life of the affected individuals, it also lessens the long-term burden on health care systems.

By enabling better disease control and facilitating preventive strategies, widespread HPLC availability can play a transformative role in public health, especially in regions with a high prevalence of Hb disorders. Ensuring equitable access to this diagnostic tool is a vital step toward comprehensive thalassemia care and effective disease prevention.

Early and accurate detection can lead to timely interventions, potentially reducing the number of patients who become transfusion-dependent. In the long-term, this can significantly enhance patient outcomes and strengthen overall health care delivery for those affected.

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## REFERENCES

1. Higgins T, Schnabl K, Savoy M, Rowe P, Flamini M, Bananda S. A novel double heterozygous, HbD Punjab/HbQ India, hemoglobinopathy. Clin Biochem. 2012;45(3):264-6.
2. Singh V, Biswas AK, Baranwal AK, Asthana B, Dahiya T. Prevalence of hemoglobinopathies using high-performance liquid chromatography as diagnostic tool in anemic patients of tertiary care center of Western India. Asian J Transfus Sci. 2024;18(2):257-63.
3. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008;86(6):480-7.
4. Dharmarajan S, Pawar A, Bhide P, Kar A. Undiagnosed haemoglobinopathies among pregnant women attending antenatal care clinics in Pune, India. J Community Genet. 2021;12(3):337-44.
5. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12.
6. Balgir RS. The burden of haemoglobinopathies in India and the challenges ahead. Curr Sci. 2000;79(11):1536-47.
7. Rees DC, Brousse VA. Sickle cell disease: status with particular reference to India. Indian J Med Res. 2016;143(6):675-7.
8. Chinnaiyan S, Sylvia J, Kothandaraman S, Palanisamy B. Quality of life and thalassemia in India: a scoping review. J Family Med Prim Care. 2024;13(4):1183-90.
9. Ministry of Health & Family Welfare (Government of India), National Health Mission. Guidelines on Prevention and Control of Haemoglobinopathies in India. New Delhi: MoHFW; 2016.
10. Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: a 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016;10(1):105-10.
11. Pathak V, Sharma I, Agrawal A, Mund P. Prevalence of hemoglobinopathies detected by high performance liquid chromatography in tertiary care centre, Kota, Rajasthan. Int J Adv Med. 2024;11(5):476-9.
12. Sahoo S, Sahu N, Das P, Senapati U. Effect of megaloblastic anemia on hemoglobin A2 and diagnosis of β-thalassemia trait. Indian J Pathol Microbiol. 2023;66(2):327-31.
13. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta thalassemia: a common and clinically diverse disorder. Indian J Med Res. 2011;134(4):522-31.
14. Sahu P, Purohit P, Mantri S, Tudu R, Nayak J, Agrawalla SK, et al. Spectrum of hemoglobin disorders in southern Odisha, India: a hospital based study. Porto Biomed J. 2021;6(1):e126.
15. Shanthala Devi AM, Rameshkumar K, Sitalakshmi S. Hb D: A not so rare hemoglobinopathy. Indian J Hematol Blood Transfus. 2016;32(Suppl 1):S294-8.
16. Mohanty D, Mukherjee MB, Colah RB, Wadia M, Ghosh K, Chottray GP, et al. Spectrum of hemoglobinopathies among the primitive tribes: a multicentric study in India. Asia Pac J Public Health. 2015;27(2):NP562-71.
17. Verma S, Bhargava M, Mittal SK, Gupta R. Homozygous delta-beta thalassemia in a child: a rare cause of elevated fetal hemoglobin. Iran J Ped Hematol Oncol. 2013;3(1):222-7.
18. Balgir RS. Hereditary persistence of foetal haemoglobin in a tribal family of Orissa, India. Natl Med J India. 2004;17(3):138-40.
19. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, et al. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. J Community Genet. 2013;4(1):33-42.
20. Philip J, Sarkar RS, Kushwaha N. Microcytic hypochromic anemia: should high performance liquid chromatography be used routinely for screening anemic and antenatal patients? Indian J Pathol Microbiol. 2013;56(2):109-13.
21. Harthoorn-Lasthuizen EJ, Lindemans J, Langenhuijsen MM. Influence of iron deficiency anaemia on haemoglobin A2 levels: possible consequences for beta-thalassaemia screening. Scand J Clin Lab Invest. 1999;59(1):65-70.
22. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010;5:11.