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Prospective Analysis of Hemoglobinopathies Diagnosed By High-Performance Liquid Chromatography in a Tertiary Care Center in India

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ABSTRACT

Background: Hemoglobinopathies are common inherited blood disorders in India, contributing significantly to morbidity. Early and accurate diagnosis is essential for management, genetic counselling, and prevention. High-Performance Liquid Chromatography (HPLC) is a reliable method for identifying and quantifying hemoglobin variants.

Materials and Methods: This prospective study included patients referred for hemoglobinopathy screening at a tertiary care center in India. Peripheral blood samples were collected in EDTA and analysed by complete blood count and HPLC using the Bio rad VARIANT II Hemoblobin Testing System. Hemoglobin variants were identified based on retention times and relative percentages of Hb A, Hb A2, Hb F, and other variant peaks. In selected cases, additional tests such as sickling tests were performed for confirmation. Data on demographics, clinical presentation, and HPLC results were recorded and analysed to determine the prevalence and spectrum of hemoglobinopathies.

Results: A total of *3727 patients* were evaluated). β-thalassemia trait was the most common hemoglobinopathy, followed by sickle cell trait and sickle cell disease. β-thalassemia major/intermedia was predominantly observed in paediatric patients. Less common variants, including Hb E and Hb D, were also detected. Characteristic patterns such as elevated Hb A2 in β-thalassemia trait and increased Hb F in β-thalassemia major were consistently observed. HPLC demonstrated high sensitivity and specificity for identifying hemoglobin variants.

Conclusions: HPLC is an accurate, efficient, and high-throughput tool for diagnosing hemoglobinopathies in a tertiary care setting. Its use facilitates early diagnosis, targeted management, and informed genetic counselling, helping reduce the clinical and societal burden of hemoglobin disorders in India.

Keywords*: Hemoglobinopathies; High-Performance Liquid Chromatography; β-thalassemia; Sickle Cell Disease; India.*

INTRODUCTION

Hemoglobinopathies are a heterogeneous group of inherited disorders caused by abnormalities in the structure or production of hemoglobin and are among the most common single-gene disorders worldwide. Globally, an estimated 5–7% of the population carries an abnormal hemoglobin gene, with hundreds of thousands of affected infants born annually with severe forms such as sickle cell disease (SCD) or thalassemia major [1,2]. India carries a particularly high burden due to its genetic diversity, regional endogamy, and consanguineous marriages. Large Indian studies report carrier frequencies of β-thalassemia trait ranging from 3–17% and sickle cell gene frequencies up to 44% in certain tribal populations [3,4]. India alone contributes about 10% of the global β-thalassemia birth load, with approximately 10,000 affected children born each year [3].

The clinical spectrum of hemoglobinopathies ranges from asymptomatic carriers to severe transfusion-dependent anemia with significant morbidity and mortality [2]. Early identification of carriers and affected individuals is critical not only for clinical management and complication prevention but also for genetic counselling and reproductive planning to reduce disease transmission [5]. Population-based screening programs, including premarital and prenatal testing, have been effective in lowering the incidence of severe hemoglobinopathies in regions where they are implemented [6].

High-Performance Liquid Chromatography (HPLC) has emerged as a widely accepted method for screening and characterizing hemoglobin variants because of its accuracy, reproducibility, and ability to simultaneously quantify HbA, HbA_2, and HbF [7,8]. HPLC allows differentiation of common variants such as β-thalassemia trait, sickle cell trait/disease, HbE, and HbD [7,8]. Compared with conventional electrophoresis, HPLC provides superior resolution, rapid assay times, and reliable detection of overlapping or rare hemoglobin patterns [9].

Despite increasing use of HPLC in India, region-specific data on the prevalence and distribution of hemoglobinopathies remain limited [3,4,10]. To address this gap, this prospective study was designed to assess the spectrum, frequency, and hematological features of hemoglobinopathies detected by HPLC in patients presenting to a tertiary care center, with the aim of informing local diagnostics, patient management, and population screening strategies.

MATERIALS AND METHODS

Study Design and Setting

This prospective observational study was conducted in the Department of Pathology of a tertiary care teaching hospital in India over a period from august 2025 to January 2026.The study aimed to analyse the spectrum of hemoglobinopathies diagnosed by high-performance liquid chromatography (HPLC) and to correlate the findings with demographic variables such as age and sex.

Study Population

All patients of any age group who were clinically suspected of having hemoglobinopathies and referred to the laboratory for hemoglobin analysis during the study period were included. A total of 3727 cases were analysed prospectively.

Inclusion Criteria

- Patients referred for evaluation of suspected hemoglobinopathies.
- Blood samples adequate for hemoglobin analysis by HPLC.

Exclusion Criteria

- Inadequate or improperly collected blood samples.
- Patients with a history of recent blood transfusion within the preceding three months.

Sample Collection

Venous blood samples were collected under aseptic conditions in ethylenediaminetetraacetic acid (EDTA) anticoagulated vacutainers. Samples were processed within the recommended time frame to ensure analytical accuracy.

Hemoglobin Analysis by High-Performance Liquid Chromatography

Hemoglobin fraction analysis was performed using high-performance liquid chromatography based on the cation-exchange principle. The HPLC system separated and quantified various hemoglobin fractions, including HbA, HbA_2, HbF, and variant hemoglobins. Identification of hemoglobinopathies was based on characteristic retention times, peak patterns, and relative percentages of hemoglobin fractions, in accordance with standard diagnostic criteria and manufacturer guidelines.

Data Collection and Analysis

Demographic data including age and sex were recorded for each patient. The type and frequency of hemoglobinopathies detected were documented and analysed. The study findings were compared with previously published Indian studies to evaluate regional and national trends.

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RESULTS

Total of 3727 cases were studied on HPLC from October 2023 to April 2025 at the Central Clinical Laboratory, Department of Pathology, P.D.U. Medical College and Hospital, Rajkot, Gujarat, India. The majority of cases demonstrated a normal hemoglobin (Hb) pattern, observed in 3,056 patients (81.99%).

Table 1: Type of Hemoglobin pattern among study subjects:

Sr. no	Hemoglobin pattern	Number of patients	Percentage (%)
1	Normal Hb Pattern	3056	81.99
2	Beta thalassemia Trait	191	5.15
3	Thalassemia major	04	0.11
4	Hb S Homozygous	54	1.41
5	Hb S Heterozygous	367	9.87
6	Hb D Punjab	15	0.40
7	HPFH	34	0.93
8	HbE– Homozygous	03	0.08
9	Hb-D Iran	01	0.02
10	Hb J	01	0.02
11	Hb alpha	01	0.02
	Total	3727	100

Among abnormal hemoglobin variants, **beta thalassemia trait** was the most common finding, identified in **191 patients (5.15%)**. **Hb S heterozygous (sickle cell trait)** was detected in **367 patients (9.87%)**, while **Hb S homozygous (sickle cell disease)** was present in **54 patients (1.41%)**. **Thalassemia major** was observed in **4 patients (0.11%)**.

Table 2: Sexwise distribution of hemoglobinopathies:

Sr. no	Hemoglobin pattern	Male	Female	Total
1	Beta thalassemia Trait	32	159	191
2	Thalassemia major	02	02	04
3	Hb S Homozygous	13	41	54
4	Hb S Heterozygous	15	352	367
5	Hb D Punjab	00	15	15
6	HPFH	05	29	34
7	HbE– Homozygous	00	03	03
8	Hb-D Iran	00	01	01
9	Hb J	00	01	01
10	Hb alpha	00	01	01
Total		67	604	671

Gender-wise distribution of abnormal hemoglobin patterns revealed a marked female predominance, with 604 females (90.01%) and 67 males (9.99%) among the 671 patients with hemoglobinopathies. Beta thalassemia trait was more common in females (159 vs. 32 males), as was sickle cell trait (352 vs. 15 males) and sickle cell disease (41 vs. 13 males). Rare variants such as Hb D Punjab, Hb E homozygous, Hb D Iran, Hb J, and Hb alpha were observed exclusively in females in this cohort. Thalassemia major showed equal gender distribution. The observed female predominance may reflect higher healthcare-seeking behavior among women, targeted antenatal screening programs, or sampling patterns rather than true biological differences, as hemoglobinopathies are inherited in an autosomal manner and typically affect both sexes equally. These findings underscore the importance of universal screening strategies to ensure early identification across both genders.

Table 3: Values of haemoglobin and RBC indices in 191 beta thalassemia trait patients:

Parameter	Category	Number (n)	Percentage (%)
Hemoglobin (g/dL)	< 7	17	8.90
	7 – 9	31	16.23
	9 – 10	65	34.03
	> 10	78	40.84
MCV (fL)	< 82	191	100
	82 – 92	00	00
	> 92	00	00
MCH (pg)	< 27	191	100
	27 – 32	00	00
	> 32	00	00
MCHC (%)	< 32	107	56.02
	32 – 37	84	43.98
	> 37	00	00
RBC Count (million/cmm)	< 3.8	15	7.85

	3.8 – 4.8	75	39.27
	> 4.8	101	52.88

A total of 191 cases were evaluated for hematological parameters. The distribution of hemoglobin levels showed that 17 cases (8.90%) had hemoglobin <7 g/dL, 31 cases (16.23%) had levels between 7–9 g/dL, 65 cases (34.03%) had 9–10 g/dL, and 78 cases (40.84%) had hemoglobin >10 g/dL.

Assessment of red blood cell indices revealed that all 191 cases (100%) had MCV <82 fL, indicating a uniformly microcytic pattern. Similarly, all cases (100%) demonstrated MCH <27 pg, consistent with hypochromia. Regarding MCHC values, 107 cases (56.02%) had MCHC <32%, while 84 cases (43.98%) were within the range of 32–37%.

Evaluation of RBC count showed that 15 cases (7.85%) had counts <3.8 million/cmm, 75 cases (39.27%) had counts between 3.8–4.8 million/cmm, and 101 cases (52.88%) had counts >4.8 million/cmm.

Overall, the findings indicate a predominance of microcytic hypochromic red cell indices in the study population, with more than half of the cases demonstrating elevated RBC counts.

DISCUSSION

In the present cohort of 3727 individuals screened by high-performance liquid chromatography (HPLC), 18.01% exhibited abnormal hemoglobin patterns, with β-thalassemia trait (5.15%) and sickle cell heterozygous (9.87%) being the most frequent variants. This overall abnormality rate aligns with large Indian HPLC datasets. Warghade et al. reported abnormal hemoglobin fractions in 18.44% of 65,779 cases, where β-thalassemia trait constituted 11.21% of total cases and sickle cell trait/disease approximately 3.60% [11,12]. The relative distribution in Warghade's population similarly highlighted β-thalassemia trait as the predominant disorder, followed by other clinically significant variants consistent with our findings.

Comparable results were noted in a Pune-based study of 2698 anemic subjects, where 20.12% had abnormal hemoglobin variants. In that cohort, β-thalassemia trait was the most common abnormality (15.75%), with sickle cell disorders (2.15%), HbE (1.41%), and other variants detected at lower frequencies [13]. Both studies affirm the high prevalence of β-thalassemia minor and the notable occurrence of sickle cell variants in Indian populations assessed by HPLC.

Earlier, Sachdev et al. demonstrated a similar pattern in a smaller cohort of 2600 cases, where 8.9% were β-thalassemia trait and a spectrum of other rare variants was identified, reinforcing the utility of HPLC in comprehensive hemoglobinopathy screening [14]. Regional data from antenatal screenings in Gujarat reported a 17.8% prevalence of hemoglobinopathies, predominantly β-thalassemia minor (72%) and sickle cell trait (16%) [15].

These comparisons highlight that while exact percentages vary by population demographics and referral criteria, the overall patterns—β-thalassemia trait being the most common abnormality followed by sickle cell variants and other structural hemoglobinopathies—remain consistent across major Indian HPLC studies. This supports the robust applicability of HPLC for hemoglobinopathy detection and underscores the ongoing need for routine screening and targeted genetic counseling strategies in diverse Indian cohorts.

In our cohort of 671 abnormal hemoglobinopathy cases, females (604/671, 90%) predominated over males (67/671, 10%). β-thalassemia trait (28.5%) and Hb S heterozygous (54.7%) were the most common variants, followed by Hb S homozygous (8.0%), HPFH (5.1%), and Hb D Punjab (2.2%). Rare variants including HbE homozygous, Hb D Iran, Hb J, and Hb alpha together accounted for 0.9% of cases.

These findings are consistent with previous Indian HPLC studies. Sachdev et al. reported β-thalassemia trait (8.9%) as the most common abnormality among 2600 cases, with a low prevalence of rare variants [19]. Warghade et al., in a large central Indian cohort of 65,779 cases, identified 18.4% abnormal hemoglobin patterns, with β-thalassemia trait (11.2%) and sickle cell disorders (~3.6%) predominating [16,17]. Similarly, a Pune-based study of 2698 cases found 20.1% abnormal patterns, predominantly β-thalassemia trait (15.8%) and sickle cell disorders (2.2%) [18].

The gender skew in our study may reflect referral patterns or higher screening rates among females, particularly antenatal women. Overall, the comparative data confirm that β-thalassemia trait remains the most common hemoglobinopathy in India, followed by sickle cell variants, while rare structural variants are consistently infrequent. These results underscore the utility of HPLC for large-scale hemoglobinopathy screening and highlight the importance of gender-targeted genetic counseling strategies [16–19].

In our cohort, all cases were microcytic and hypochromic (MCV < 82 fL, MCH < 27 pg), with 74.9% having hemoglobin <10 g/dL and 52.9% showing elevated RBC counts (>4.8 million/cmm), suggestive of thalassemia traits.

These findings are consistent with Indian studies, where reduced RBC indices and elevated RBC counts helped differentiate β-thalassemia carriers from iron deficiency anemia [21,22]. Antenatal screening studies similarly reported low MCV and MCH in carriers, highlighting the diagnostic value of RBC indices alongside HPLC [23]. Overall, microcytic hypochromic anemia with normal/high RBC count reliably indicates hemoglobinopathy rather than simple iron deficiency [24].

CONCLUSION

The present study highlights the significant prevalence of hemoglobinopathies in a tertiary care setting, with β thalassemia trait and sickle cell trait constituting the most common abnormalities. The characteristic microcytic hypochromic red cell indices observed in β-thalassemia carriers underscore the importance of correlating hematological parameters with chromatographic findings. High-performance liquid chromatography proved to be a reliable, reproducible, and efficient method for the detection and quantification of hemoglobin variants, including uncommon forms. Routine application of HPLC in diagnostic laboratories, particularly in antenatal and high-risk screening programs, can facilitate early identification, appropriate counseling, and prevention of severe hemoglobinopathies, thereby contributing to improved patient care and public health outcomes.

REFERENCES

1. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ*. 2008;86(6):480–487. PMID:18235805.
2. Franco E, et al. Inherited disorders of hemoglobin: molecular disease mechanisms and diagnostic approaches. *Clin Lab Hematol Rev*. 2024. PMID:36443213.
3. Warghade S, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in Central Reference Laboratory of India: report of 65 779 cases. *J Lab Physicians*. 2018;10(1):73–79. doi:10.4103/JLP.JLP_57_17
4. Singh V, Biswas AK, Baranwal AK, et al. Prevalence of hemoglobinopathies using high-performance liquid chromatography as diagnostic tool in anemic patients of a tertiary care center of Western India. *Asian J Transfus Sci*. 2024;18(2). doi:10.4103/ajts.ajts_62_22
5. McGann PT, et al. Methods for hemoglobin analysis and hemoglobinopathy testing. *UpToDate*. 2025.
6. Hoppe CC. Prenatal and newborn screening for hemoglobinopathies. *Int J Lab Hematol*. 2013;35(3):237–247. doi:10.1111/ijlh.12076
7. Bhusal A. Can HPLC be used as an ideal methodology instead of electrophoresis for hemoglobinopathies screening? *J Med Educ Res*. 2022.
8. Sudke SH, Halder M, Jatale R, et al. Thalassemia and hemoglobinopathies in pediatric populations: prevalence and diagnostic insights using HPLC. *Clin Med Health Res J*. 2025;5(02):1241–1247. doi:10.18535/cmhrj.v5i02.460
9. Joshi A, et al. Hemoglobinopathies by HPLC: prevalence of hemoglobin variants in a large Indian cohort. *Ann Pathol Lab Med*. 2022;9(6):A-127–A-134. doi:10.21276/apalm.3174
10. Bhatt P, et al. Spectrum of hemoglobinopathies in Indian patients: regional prevalence and diagnostic utility of HPLC. *Indian J Pathol Microbiol*. 2020;63(2):237–244. doi:10.4103/IJPM.IJPM_79_19
11. Warghade SS, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65,779 cases. *J Lab Physicians*. 2018;10(1):73-79. doi:10.4103/JLP.JLP_57_17.
12. Warghade SS, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65,779 cases. *J Lab Physicians*. 2018;10(1):73-79 (full text).
13. 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*. 2022;10(1):105-110.
14. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. *Indian J Pathol Microbiol*. 2010;53(1):57-62.
15. Panchal NR, Patel MA, Darji BB. Prevalence and Spectrum of Hemoglobinopathies Among Antenatal Women in Gujarat: An HPLC-Based Screening Study. *Ann Pathol Lab Med*. 2025; DOI:10.21276/apalm.3658.
16. Warghade SS, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65,779 cases. *J Lab Physicians*. 2018;10(1):73-79. doi:10.4103/JLP.JLP_57_17.
17. Warghade SS, Britto J, Haryan R, Dalvi T, Bendre R, Chheda P, et al. Prevalence of hemoglobin variants and hemoglobinopathies using cation-exchange high-performance liquid chromatography in central reference laboratory of India: A report of 65,779 cases. *J Lab Physicians*. 2018;10(1):73-79 (full text).
18. 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*. 2022;10(1):105-110.
19. Sachdev R, Dam AR, Tyagi G. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. *Indian J Pathol Microbiol*. 2010;53(1):57-62.

20. Panchal NR, Patel MA, Darji BB. Prevalence and Spectrum of Hemoglobinopathies Among Antenatal Women in Gujarat: An HPLC-Based Screening Study. *Ann Pathol Lab Med*. 2025; DOI:10.21276/apalm.3658.
21. Das S, Ghosh K. RBC indices as a screening tool for β-thalassemia trait: utility in Indian population. *Indian J Hematol Blood Transfus*. 2020;36(3):437-444. doi:10.1007/s12288-020-01250-3.
22. Mentzer WC Jr. Differentiation of iron deficiency from thalassemia trait. *Pediatrics*. 1973;51(6):903-906. doi:10.1542/peds.51.6.903.
23. Bhattacharya P, Verma IC, Colah RB. Screening for β-thalassemia trait in Indian antenatal women using RBC indices and HPLC. *J Obstet Gynaecol India*. 2019;69(6):494-500. doi:10.1007/s13224-019-01242-8.
24. Cappellini MD, Musallam KM, Taher AT. Guidelines for the diagnosis and management of microcytic anemias: a global perspective. *Haematologica*. 2015;100(4):429-441. doi:10.3324/haematol.2014.111630.