Section: Miscellaneous

Original Research Article

HPLC-BASED EVALUATION OF HEMOGLOBINOPATHIES IN PEDIATRIC POPULATION: A RETROSPECTIVE CROSS-SECTIONAL STUDY FROM A TERTIARY CARE CENTER IN SOUTH INDIA

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Received *: 27/01/2026*
Received in revised form *: 15/03/2026*
Accepted *: 01/04/2026*

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DOI: 10.70034/ijmedph.2026.2.64

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (2); 388-394

ABSTRACT

Background: Hemoglobinopathies are among the most common inherited disorders worldwide and contribute significantly to pediatric morbidity, particularly in developing countries like India. Early detection using reliable diagnostic modalities is essential for timely intervention and genetic counseling. The objective is to evaluate the prevalence and pattern of hemoglobinopathies in pediatric patients using High-Performance Liquid Chromatography (HPLC) and to analyze their hematological characteristics.

Materials and Methods: This retrospective cross-sectional study included 150 pediatric patients (0–14 years) over a period of 12 months (January–December 2025). Blood samples collected in EDTA were analyzed using an automated hematology analyzer for red cell indices and BIORAD D-10 HPLC system for hemoglobin variant detection. Cases were categorized based on hemoglobin fractions and retention times.

Results: Out of 150 cases, 109 (72.7%) showed normal hemoglobin pattern, while 36 (24.0%) had hemoglobinopathies and 5 (3.3%) were indeterminate. Among abnormal cases: Sickle cell disease (HbSS): 11 cases (7.3%), Beta thalassemia trait: 7 cases (4.7%), Sickle cell trait: 6 cases (4%) & Compound heterozygous states: 6 cases (4%). A slight male predominance (52.7%) was observed. The most affected age group was 6–10 years (38.9%). Approximately 80% of cases showed microcytic hypochromic indices.

Conclusion: Hemoglobinopathies, particularly sickle cell disease and beta thalassemia trait, are prevalent in the pediatric population. HPLC is a reliable and effective tool for screening and diagnosis. Early detection combined with genetic counseling and preventive strategies is essential to reduce disease burden.

Keywords: Hemoglobinopathies, Sickle Cell Disease, Beta Thalassemia Trait, High-Performance Liquid Chromatography (HPLC), Pediatric Anemia, Hemoglobin Variants, Red Cell Indices, Screening.

INTRODUCTION

Hemoglobinopathies constitute a heterogeneous group of inherited disorders characterized by either abnormal synthesis or structural alterations of the hemoglobin molecule, leading to significant clinical and public health implications. These disorders represent a major cause of morbidity and mortality globally, particularly in low- and middle-income countries, with India bearing a substantial burden due to its genetic diversity and high carrier frequencies.[1] In India, the most prevalent hemoglobinopathies include β-thalassemia major, β-thalassemia trait, and sickle cell disease (SCD). The carrier frequency of sickle cell hemoglobin varies widely across different regions, ranging from 1% to as high as 35%,

particularly affecting tribal populations in central and southern India.[2] Globally, it is estimated that approximately 7% of the population carries abnormal hemoglobin genes, with nearly 300,000 to 500,000 newborns affected annually by clinically significant hemoglobin disorders. Among these, sickle cell syndromes account for nearly 70% of cases, while thalassemias comprise the remaining proportion.[3]

Hemoglobinopathies follow an autosomal recessive inheritance pattern. Homozygous states or compound heterozygous combinations result in clinically significant phenotypes with varying severity, including thalassemia major, thalassemia intermedia, sickle cell disease, and hemoglobin E syndromes. In contrast, heterozygous carriers are typically asymptomatic but exhibit characteristic hematological parameters that facilitate screening and early detection.[4]

The genetic complexity of hemoglobinopathies is further amplified by the presence of multiple hemoglobin variants and thalassemia mutations, which may co-inherit and interact to produce diverse and often atypical hematological phenotypes. Accurate diagnosis in such cases requires a combination of hematological indices, high-performance liquid chromatography (HPLC), family studies, and molecular genetic analysis.[5]

High-performance liquid chromatography (HPLC) has emerged as a reliable, rapid, and reproducible technique for the detection and quantification of hemoglobin variants. It plays a crucial role in screening, diagnosis, and epidemiological studies of hemoglobinopathies, particularly in pediatric populations where early detection can significantly influence management and genetic counseling.[6]

Aim & Objectives

To evaluate the prevalence and pattern of hemoglobinopathies in pediatric patients using High-Performance Liquid Chromatography (HPLC) and to analyze their hematological characteristics.

MATERIALS AND METHODS

Study Design: This study was designed as a retrospective cross-sectional study.

Study Setting and Duration: The study was conducted in a central research laboratory over a period of 12 months (January 2025 to December 2025).

Sample Size: A total of 150 pediatric blood samples received for hemoglobin variant analysis were included in the study.

Study Population: Pediatric patients aged 0–14 years who were referred for evaluation of suspected hemoglobinopathies.

Inclusion Criteria

- Children aged 0–14 years with clinical suspicion of hemoglobinopathy
- Pediatric cases with family history of hereditary hemoglobin disorders
- Cases referred for evaluation of unexplained anemia or abnormal red cell indices

Exclusion Criteria

- Age >14 years
- History of recent blood transfusion (within last 3 months)
- Previously diagnosed cases of hemoglobinopathies under treatment or follow-up

Sample Collection: Venous blood samples were collected in EDTA anticoagulated vacutainers under aseptic conditions.

Hematological Analysis: Complete blood count (CBC) and red cell indices were analyzed using a fully automated hematology analyzer (Mindray BC-780), ensuring calibration and internal quality control prior to analysis.

HPLC Analysis: All samples were subjected to hemoglobin variant analysis using BIORAD D-10 HPLC system, employing the β-thalassemia short program.

- Two levels of BIORAD controls were run daily for quality assurance
- Hemoglobin fractions were identified based on retention time and peak characteristics
- Quantification of HbA, HbA2, HbF, and abnormal variants (HbS, HbE, etc.) was performed
- Chromatograms were generated and interpreted according to manufacturer guidelines and standard diagnostic criteria

Data Interpretation

Hemoglobin variants were categorized based on:

- Percentage of hemoglobin fractions
- Retention time windows
- Correlation with red cell indices

Where required, findings were correlated with clinical history and family background to support diagnosis.

RESULTS

A total of 150 pediatric samples were analyzed during the study period. Among these, 109 cases (72.7%) demonstrated a normal hemoglobin pattern. Hemoglobinopathies were identified in 36 cases (24.0%), while 5 cases (3.3%) were categorized as indeterminate.

Among the 36 confirmed hemoglobinopathy cases, 19 (52.7%) were males and 17 (47.3%) were females. The mean age of affected males was 6.8 years, and females 4.3 years. The most common age group for presentation was 6–10 years.

Table 1: Distribution Pattern of Hemoglobin Variants by HPLC

Hemoglobin Pattern	Number of Cases (n)	Percentage (%)
Normal Hb Pattern	109	72.7
Sickle Cell Disease (HbSS)	11	7.3
Beta Thalassemia Trait (BTT)	7	4.7
Sickle Cell Trait (SCT)	6	4.0
Compound Heterozygous (HbS + β-thalassemia)	6	4.0
Indeterminate	5	3.3
Beta Thalassemia Major (BTM)	1	0.7
Beta Thalassemia Intermedia (BTI)	1	0.7
Borderline HbA2	1	0.7
HPFH Trait	1	0.7
Elevated HbF	1	0.7
Elevated HbC	1	0.7
Total	150	100

The overall prevalence of hemoglobinopathies in the study population was 24%, with sickle cell disease (HbSS) being the most common variant (7.3%), followed by beta thalassemia trait (4.7%). Compound heterozygous states and sickle cell trait were also observed with equal frequency (4%). Rare variants such as HPFH, elevated HbF, and HbC were detected in isolated cases, highlighting the heterogeneity of hemoglobin disorders.

Table 2: Gender Distribution of Hemoglobinopathy Cases (n = 36)

Gender	Number of Cases	Percentage (%)
Males	19	52.7
Females	17	47.3
Total	36	100

A slight male predominance was observed among hemoglobinopathy cases, although the distribution was nearly equal, suggesting no significant gender predisposition, consistent with the autosomal recessive inheritance pattern.

Table 3: Age-wise Distribution of Hemoglobinopathies (n = 36)

Age Group (Years)	SCH (HbSS)	SCT	BTM	BTI	BTT	Borderline HbA2	Compound (HbS+β-thal)	HPFH	Elevated HbF	Elevated HbC	Total
<1	0	0	0	0	2	0	0	1	0	1	4
1–5	5	3	1	1	1	0	1	0	1	0	13
6–10	5	2	0	0	2	1	4	0	0	0	14
11–14	1	0	0	0	2	0	1	0	0	0	4
Total	11	6	1	1	7	1	6	1	1	1	36

The highest number of hemoglobinopathy cases (38.9%) were observed in the 6–10 years age group, followed by the 1–5 years group. This suggests that clinical manifestation and diagnosis are most common during early school-age years. Neonatal and infant cases were relatively fewer, indicating possible under-screening or delayed diagnosis.

Pattern Analysis: The most common hemoglobinopathy identified was sickle cell disease (HbSS) (7.3%), followed by beta thalassemia trait (4.7%).

HbSS:

- Males: 5 (45.5%)
- Females: 6 (54.5%)

BTT:

- Males: 4 (57.1%)
- Females: 3 (42.9%)

Table 4: Hematological Profile of Sickle Cell Disease (HbSS) Cases (n = 11)

No.	Age (yrs)	Gender	HbA (%)	HbF (%)	HbA2 (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbS (%)
1	4	F	15.0	24.9	1.8	83.4	23.9	28.7	53.0
2	7	F	10.5	18.2	2.3	77.4	24.0	31.2	69.0
3	10	M	14.0	20.5	3.9	74.2	21.4	28.8	61.6
4	6	F	14.5	20.3	2.2	76.7	22.8	29.8	63.0
5	3	M	8.3	18.3	1.6	81.8	24.6	29.8	71.8
6	10	M	7.0	21.5	1.6	105.3	34.3	32.7	69.9
7	5	F	6.7	18.3	2.7	96.1	29.1	30.3	72.3
8	7	M	43.4	27.4	0.8	91.4	26.2	28.6	55.8
9	4	F	5.2	16.4	1.8	78.2	22.4	28.6	76.6
10	11	M	8.6	16.3	2.2	96.6	31.7	32.8	72.9
11	3	F	13.5	16.2	3.2	66.1	20.4	30.9	67.1

All HbSS cases showed elevated HbS (55–76%) with increased HbF levels, consistent with disease phenotype. HbA was generally low; however, one case showed relatively higher HbA (43.4%), which may indicate:

- Recent transfusion history (despite exclusion)
- Laboratory variation
- Misclassification or compound state

This case requires clinical correlation and review of transfusion history.

Table 5: Hematological Profile of Beta Thalassemia Trait (BTT) Cases (n = 7)

No.	Age (yrs)	Gender	HbA (%)	HbF (%)	HbA2 (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
1	14	M	93.1	1.0	5.9	69.5	19.9	28.5
2	1	F	92.8	2.3	4.9	56.0	16.8	30.1
3	0.7	F	92.2	3.0	4.6	57.8	16.4	28.4
4	11	M	91.3	1.6	7.1	66.1	19.4	29.5
5	7	F	93.5	0.8	5.7	72.5	19.8	27.3
6	0.6	M	91.5	4.3	4.2	47.4	13.7	28.8
7	1	M	91.1	5.1	3.8	52.3	12.6	24.2

All cases demonstrated microcytic hypochromic indices (low MCV and MCH) with elevated HbA2 (>3.5%), confirming beta thalassemia trait.

One case with HbA2 = 3.8% was borderline but consistent with BTT when correlated with red cell indices.

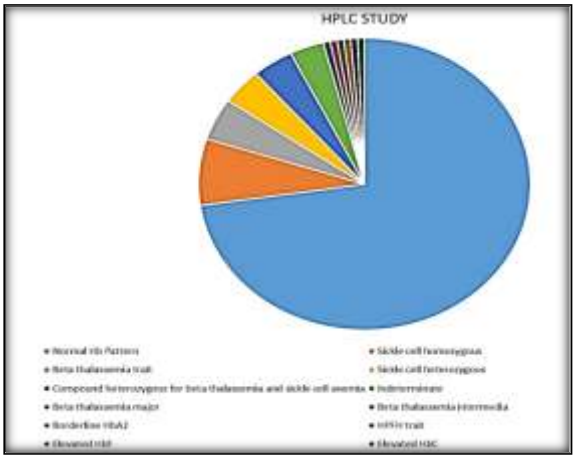

Figure 1: Distribution of hemoglobin variants detected by HPLC among pediatric cases (n = 150) showing predominance of normal hemoglobin pattern (72.7%) with significant proportion of sickle cell disease and thalassemia variants.

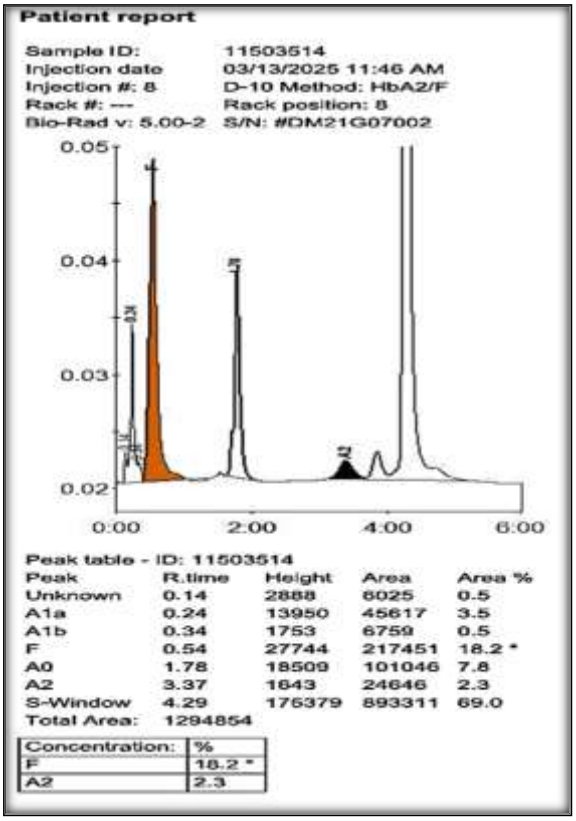

Figure 2: HPLC chromatogram of a case showing elevated HbF (18.2%) with normal HbA2 (2.3%), suggestive of increased fetal hemoglobin pattern.

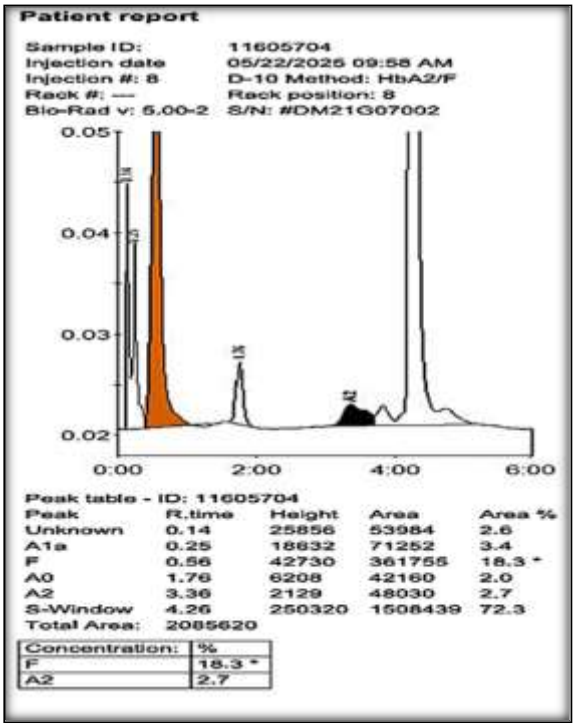

Figure 3: HPLC chromatogram demonstrating sickle cell disease pattern with prominent HbS peak (72.3%) and elevated HbF (18.3%), consistent with HbSS phenotype.

Figure 4: Peripheral smear image showing sickled red blood cells demonstrating characteristic elongated and crescent-shaped morphology.

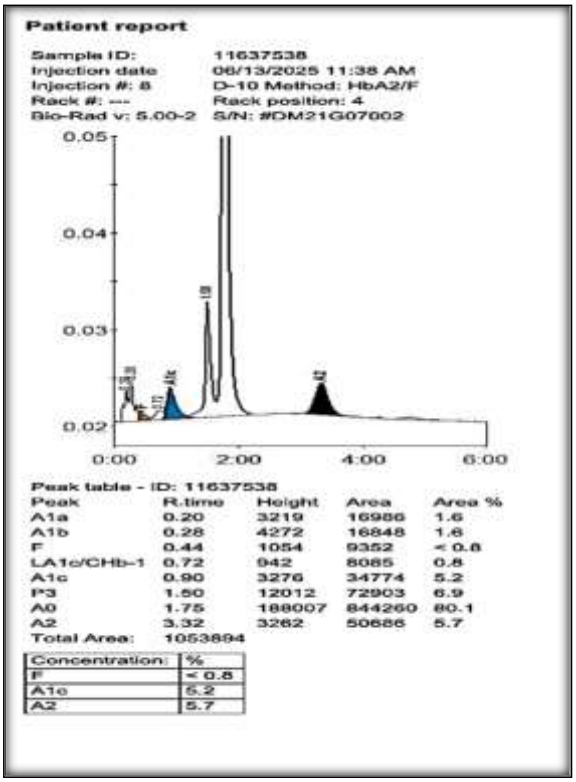

Figure 5: HPLC chromatogram showing elevated HbA2 (5.7%) with reduced HbA, consistent with beta thalassemia trait.

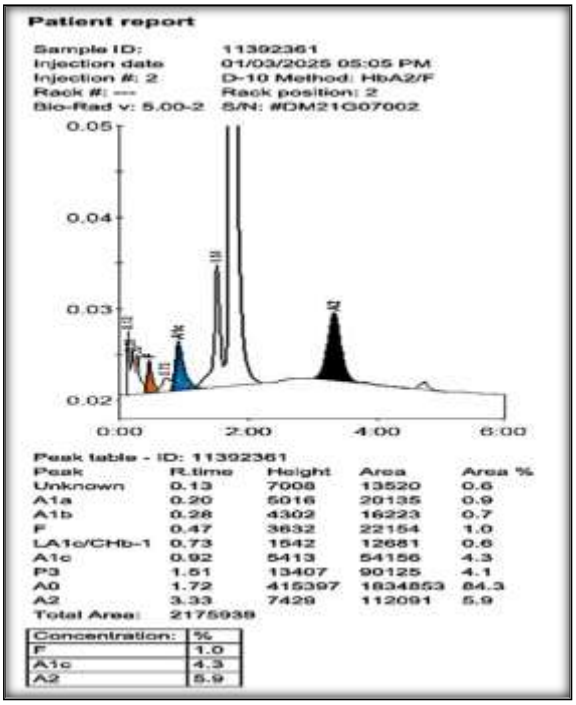

Figure 6: HPLC chromatogram demonstrating increased HbA2 (5.9%) with normal HbF levels, further supporting diagnosis of beta thalassemia trait.

DISCUSSION

Hemoglobinopathies are inherited disorders resulting from defects in globin chain synthesis or structure, broadly categorized into quantitative defects (thalassemias) and qualitative defects (structural

hemoglobin variants such as sickle cell disease). These conditions contribute significantly to pediatric anemia and remain a major public health concern in India.

In the present study comprising 150 pediatric cases, a normal hemoglobin pattern was observed in 109 cases (72.7%), while 36 cases (24.0%) demonstrated hemoglobinopathies and 5 cases (3.3%) were indeterminate. This distribution is comparable to findings reported by Sudke et al,[7] who documented a predominance of normal hemoglobin patterns with a significant proportion of abnormal variants in pediatric populations. Similarly, Mondal et al,[9] reported a comparable prevalence of hemoglobinopathies in children, supporting the consistency of disease burden across different Indian regions.

A slight male predominance (52.7% males vs 47.3% females) was observed among hemoglobinopathy cases in the present study. This trend is in agreement with observations by Dhar et al,[10] and Saba et al,[11] although the difference is minimal and does not suggest true gender predisposition, consistent with the autosomal recessive inheritance pattern of these disorders.

Age-wise analysis revealed that the maximum number of cases (38.9%) occurred in the 6–10 years age group, followed closely by the 1–5 years group (36.1%). This indicates that hemoglobinopathies are more frequently diagnosed during early childhood, likely due to increasing clinical manifestations and healthcare evaluation during this period. Similar age distribution patterns have been reported by Garg et al,[12] who observed higher detection rates in children beyond infancy, emphasizing delayed clinical recognition and referral.

In the present study, sickle cell disease (HbSS) was the most common hemoglobinopathy, accounting for 11 cases (7.3%), followed by beta thalassemia trait (7 cases; 4.7%). These findings are comparable to those reported by Sanghavi et al,[13] who also identified sickle cell disorders as a predominant variant in certain Indian populations. Additionally, sickle cell trait (4%) and compound heterozygous states (HbS + β-thalassemia; 4%) were observed, reflecting the genetic heterogeneity and co-inheritance patterns prevalent in the study population.

The detection of borderline HbA2 levels (0.7%) in this study highlights a critical diagnostic challenge. Colaco et al,[14] emphasized that borderline HbA2 values may represent silent carriers or coexisting conditions and require further evaluation using molecular techniques. In the present study, such cases were interpreted in conjunction with red cell indices to enhance diagnostic accuracy.

A notable proportion of indeterminate cases (3.3%) was observed, particularly in children below one year of age. This can be attributed to the dynamic changes in hemoglobin fractions during infancy, where fetal hemoglobin predominates and gradually transitions to adult hemoglobin. Such variability can limit the diagnostic reliability of HPLC in early life, necessitating follow-up testing for definitive diagnosis.

Evaluation of hematological parameters revealed that approximately 80% of hemoglobinopathy cases exhibited reduced MCV and MCH, consistent with microcytic hypochromic anemia. Around 15% of cases showed normal indices, while 5% demonstrated elevated values, possibly due to associated nutritional deficiencies or clinical variations. These findings are in concordance with Solanki et al,[14] who reported similar alterations in red cell indices among pediatric hemoglobinopathy cases.

HPLC proved to be an effective diagnostic modality in this study, enabling accurate identification and quantification of hemoglobin variants. However, certain limitations such as borderline HbA2 values, age-related variability, and occasional indeterminate patterns highlight the need for comprehensive evaluation, including clinical correlation and, where necessary, molecular diagnostic confirmation.[15,16]

CONCLUSION

Hemoglobinopathies continue to represent a significant health burden in the pediatric population, contributing substantially to the prevalence of anemia in India. The present study demonstrates a notable regional prevalence of sickle cell disease and beta thalassemia trait, with the highest detection occurring in early childhood.

High-Performance Liquid Chromatography (HPLC) has proven to be a reliable, rapid, and reproducible tool for the screening and diagnosis of hemoglobinopathies. Its application in routine clinical practice facilitates early detection, enabling timely intervention and genetic counseling.

To effectively reduce disease burden and improve long-term outcomes, a comprehensive approach is essential. This includes:

- Population-based screening programs, particularly in high-prevalence regions
- Pre-marital and antenatal carrier screening
- Genetic counseling for at-risk couples
- Integration of prenatal diagnostic services

Early identification and preventive strategies remain the cornerstone in controlling hemoglobinopathies and improving the quality of life in affected individuals.

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