# Prevalence of Hemoglobinopathies and Hemoglobin Variants among Anaemic Children: A Cation Exchange-High Performance Liquid **Chromatography-Based Study at a Tertiary Care Teaching Hospital**

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# ABSTRACT

Background: Hemoglobinopathies are a major cause of anemia in India, particularly in Maharashtra, where diverse ethnic groups are affected. Early diagnosis is essential to reduce morbidity and mortality. High-performance liquid chromatography (HPLC) provides a reliable and superior alternative to conventional screening methods. Methods: A hospital-based cross-sectional study was conducted over 18 months at a tertiary care medical college, including 516 anemic children (Hb <12 g/dL, MCV <75 fL) aged 6 months to 12 years. Clinical history, demographic data, and laboratory investigations (CBC, reticulocyte count, peripheral smear, sickling test, solubility test, NESTROFT, HPLC) were analyzed. Statistical analysis included t-tests and chi-square tests, with significance set at p < 0.05. Results: Among 516 children, 278 (54%) were male, and 44% were aged 0.5-5 years. Consanguinity was observed in 21%, and prior transfusions in 20%. Hemoglobinopathies were identified in 70 children (13.57%), with sickle cell trait (5.62%), sickle cell disease (3.29%),  $\beta$ -thalassemia trait (2.52%), and  $\beta$ -thalassemia major (1.36%) as common variants. Prevalence was highest in the Manelwarli caste (28.57%). Hemoglobin levels were significantly lower in children with hemoglobinopathies (8.46 vs. 9.82 g/dL, p=0.0102), while MCV (72.63 vs. 63.91 fL, p<0.0001) and RDW (15.76% vs. 14.77%, p=0.0226) were higher. Transfusions were required in 71.4% of  $\beta$ thalassemia major and 41.2% of sickle cell disease cases. Screening tests showed 52-56% concordance with HPLC. **Conclusion:** Hemoglobinopathies significantly contribute to anemia, particularly among younger children and consanguineous families. HPLC provides superior diagnostic accuracy compared to traditional screening methods, enabling early detection of severe cases requiring transfusions. Routine screening and genetic counseling are crucial to reducing the disease burden.

KEYWORDS: Thalassemia, Sickle cell disease, Anemia, Pediatric, Consanguinity, Variant hemoglobin, Screening tests

# INTRODUCTION

Hemoglobinopathies are among the most prevalent inherited genetic disorders worldwide, characterized by either reduced synthesis or structural abnormalities of hemoglobin chains.<sup>[1, 2]</sup> Sickle cell disease (SCD) and thalassemia syndromes are the most common forms, leading to chronic anemia, end-organ damage, and significant healthcare burdens.<sup>[3, 4]</sup> In India, anemia is a major public health issue, especially in children, contributing to poor growth, cognitive delay, and increased morbidity. [5, 6]

According to the World Health Organization, about 5% of the global population are carriers of hemoglobinopathies, with the highest burden observed in regions such as sub-Saharan Africa, the Middle East, and South Asia.<sup>[2, 7]</sup> India alone harbors an estimated 30 million carriers, with a mean prevalence of 3.3%. [8] Beta-thalassemia trait incidence ranges from 3% to 17%, and the sickle cell gene prevalence may reach up to 44% in certain Indian regions, particularly among tribal communities.<sup>[9, 10]</sup> Maharashtra, with its diverse population, shows considerable variation in prevalence, with higher rates in tribal populations. [11]

Early diagnosis and intervention are vital for managing hemoglobinopathies. High-performance liquid chromatog-raphy (HPLC) has emerged as a sensitive and reliable tool for detecting abnormal hemoglobin variants, especially in children presenting with anemia.<sup>[12, 13]</sup> Cation-exchange HPLC offers rapid, automated, and accurate hemoglobin fractionation, making it ideal for large-scale screening.<sup>[14]</sup>

Given the genetic and clinical significance of hemoglobinopathies in the pediatric population, especially in high-prevalence areas, this study was conducted to evaluate the prevalence and spectrum of hemoglobinopathies among anemic children in a tertiary care hospital using HPLC and other hematological parameters. The findings aim to inform early screening strategies, genetic counseling, and public health planning.

The objective of this study was to assess the prevalence of hemoglobinopathies and hemoglobin variants among anemic children admitted to a tertiary care teaching hospital. Additionally, the study aimed to analyze the demographic profile and clinical presentation of the study population, evaluate their complete blood count (CBC) parameters, and provide appropriate counselling to the parents of affected children. The goal of counseling was to promote awareness, facilitate early diagnosis, and help prevent future cases through family screening and genetic guidance.

# MATERIALS AND METHODS

### **Study Design and Setting**

This was a hospital-based cross-sectional study conducted in the Department of Pathology at a tertiary care centre over 18 months, from October 2022 to April 2024. The study aimed to assess the prevalence and pattern of hemoglobinopathies among anemic children using hematological and chromatographic investigations.

### **Study Population**

Children aged 6 months to 12 years admitted to the Department of Pediatrics with clinical features of anemia were included, based on predefined criteria. Anemia was operationally defined as hemoglobin <12 g/dL and MCV <75 fL.

#### **Inclusion Criteria**

Children presenting with clinical signs of pallor or generalized weakness, Children requiring blood transfusions due to anemia, and Children with anemia and a family history of consanguinity were enrolled in the present study.

#### **Exclusion Criteria**

Children needing urgent blood transfusion at the time of enrollment, previously diagnosed cases of hemoglobinopathies admitted solely for transfusion, Neonates below 6 months of age, and Children whose parents did not provide consent were excluded from the study.

#### Sample Size and Sampling Method

A total of 516 anemic children who met the eligibility criteria were included using complete enumeration sampling.

#### **Data Collection**

Clinical and demographic data were recorded using a pre-structured proforma. Information on symptoms, past transfusions, family history, and caste distribution was collected. A thorough general and systemic examination was conducted.

#### Laboratory Investigations

Venous blood samples (2–4 mL) were collected in EDTA vials and processed for:

- Complete Blood Count (CBC) and Red Cell Indices: including Hb, RBC count, MCV, MCH, MCHC, PCV, and RDW using an automated hematology analyzer.
- Reticulocyte Count: performed using New Methylene Blue staining and manual counting under oil immersion.
- Peripheral Smear Examination: Leishman-stained smears were examined for RBC morphology.
- Sickling Test: Performed using the Sodium Metabisulfite method to detect sickle cells.
- Solubility Test: Used for detection of HbS based on turbidity in phosphate buffer with dithionite.
- NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test): Used as a screening tool for thalassemia traits.
- High Performance Liquid Chromatography (HPLC): All samples were analyzed using the BIO-RAD D-10<sup>TM</sup> Hemoglobin Testing System, which identifies and quantifies HbA, HbF, HbA2, HbS, and other variants through cation-exchange chromatography. The system operates on a 6.5-minute HbA2/F/A1c program and offers high precision for variant hemoglobin detection.

### **Ethical Considerations**

Approval for the study was obtained from the Institutional Ethics Committee prior to commencement. Written informed consent was obtained from the parents or legal guardians of all participants.

#### **Statistical Analysis**

Data were analyzed using standard statistical software. Descriptive statistics (means, standard deviations, percentages) were used for demographic and hematological data. Chi-square tests and independent t-tests were used to evaluate associations. A p-value <0.05 was considered statistically significant.

# RESULTS

Of 516 anemic children (6 months–12 years), 278 (53.88%) were male and 238 (46.12%) females, with 227 (43.99%) aged 0.5–5 years, 195 (37.79%) 6–10 years, and 94 (18.22%) 11–12 years. Consanguinity was reported in 109 (21.12%), and 103 (19.96%) had prior transfusions; among 70 children with hemoglobinopathies, 21 (30%) had consanguineous parents (Table 1).

Category	Subcate- gory	Fre- quency	Percent- age
Consanguinity (Overall, n=516)	Yes	109	21.12%
Consanguinity (Variants, n=70)	Yes	21	30.00%
Symptoms (Overall)	Pallor	325	62.98%
	Fatigue	103	19.96%
	Breath- lessness	58	11.24%
	Fever	48	9.30%
Symptoms (Among 70 cases with hemoglobinopathies)	Pallor	38	54.00%
	Fatigue	13	19.00%
	Fever	4	6.00%

### **Table 1: Baseline Characteristics of Study Population**

Pallor was the most common symptom (325, 62.98%), followed by fatigue (103, 19.96%), breathlessness (58, 11.24%), and fever (48, 9.30%). In the variant group (n=70), pallor occurred in 54% (vs. 62.98% overall, p=NS), fatigue in 19%, and fever in 6% (Table 1).

Hematological data (Table 2) showed Hb levels of 10.1-12 g/dL in 344 (66.66%), 6.1–9.0 g/dL in 111 (21.51%), and  $\leq$ 6 g/dL in 61 (11.82%). RBC count was <4.7  $\times$ 10<sup>6</sup>/ $\mu$ l in 423 (81.97%), MCV 60-75 fL in 395 (76.55%), MCH <27 pg in 336 (65.12%), MCHC <32 g/dL in 239 (46.32%), RDW >14.5% in 296 (57.36%), and PCV <30% in 349 (67.64%). Reticulocyte counts were 0.5-2.5% in 452 (87.60%) and >2.5% in 64 (12.40%). HPLC identified hemoglobinopathies in 70 children (13.57%), predominantly sickle cell trait (29, 5.62%), sickle cell disease (17, 3.29%),  $\beta$ -thalassemia trait (13, 2.52%), and  $\beta$ -thalassemia major (7, 1.36%) (Table 3). Rarer variants included S- $\beta$  thalassemia (2, 0.39%), heterozygous HbE (1, 0.19%), and SCT + HbD trait (1, 0.19%). Most cases (49, 70%) were aged 0.5-5 years, with 14% each in 6-10 and 11-12 years. Transfusions were required by 103 (19.96%) overall, with rates of 71.4% in  $\beta$ -thalassemia major, 41.2% in sickle cell disease, and 10.3% in sickle cell trait (Table 3).

Caste analysis revealed higher variant rates in Manelwarli (4/14, 28.57%), Navhi (5/21, 23.81%), Matang (2/9, 22.22%), Banjara (5/26, 19.23%), and Mahar (4/22, 18.18%) (Table 4).

Hematological parameters (Table 5) showed  $\beta$ -thalassemia major with the lowest Hb (4.97  $\pm$  1.70 g/dL) and sickle cell disease with high HbS.

Parameter	Range	Fre- quency	Percent- age
Hemoglobin (g/dL)	10.1–12	344	66.66%
	6.1–9.0	111	21.51%
	$\leq$ 6	61	11.82%
	<4.7	423	81.97%
RBC Count (10 <sup>6</sup> / $\mu$ L)	4.7–6.1	93	18.02%
	>6.1	0	0.00%
MCV (fL)	60–75	395	76.55%
	<60	121	23.44%
	<27	336	65.12%
MCH (pg)	27–31	126	24.42%
	>31	54	10.47%
	<32	239	46.32%
MCHC (g/dL)	32–36	265	51.36%
	>36	12	2.33%
	<11.5	24	4.46%
RDW (%)	11.5–14.5	197	38.18%
	>14.5	296	57.36%
	<0.5	0	0.00%
Reticulocyte Count (%)	0.5–2.5	452	87.60%
	>2.5	64	12.40%
	<20	166	32.17%
	20–25	183	35.47%
PCV (%)	25.1–30	30 115 22.2	
	30.1–35	52	10.08%
	>35.1	0	0.00%

# Table 2: Hematological Parameters of the Study Population

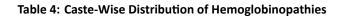
(69.8  $\pm$  9.82%) and HbF (20.98  $\pm$  1.85%). Variant vs. normal comparisons revealed lower Hb (8.46  $\pm$  1.14 vs. 9.82  $\pm$  2.56 g/dL, p=0.0102), higher MCV (72.63  $\pm$  3.15 vs. 63.91  $\pm$  6.9 fL, p<0.0001), and higher RDW (15.76  $\pm$  1.38 vs. 14.77  $\pm$  1.86, p=0.0226), with no significant differences in MCH (22.5  $\pm$  2.1 vs. 23.1  $\pm$  3.0 pg, p=0.35) or PCV (25.8  $\pm$  4.2 vs.

Hemoglobin Pattern	Cases	Required Transfu- sion	Per- cent- age of Total (n=516)	Transfu- sion Rate within Pattern
Normal	446	87	16.86%	19.51%
Sickle Cell Trait	29	3	0.58%	10.34%
Sickle Cell Disease	17	7	1.36%	41.18%
β- Thalassemia Major	7	5	0.97%	71.43%
β- Thalassemia Trait	13	0	0.00%	0.00%
S- $eta$ Thalassemia	2	0	0.00%	0.00%
Heterozygous HbE	1	0	0.00%	0.00%
SCT + HbD Trait	1	1	0.19%	100.00%
Total Variants	70	16	13.57%	22.86%

# Table 3: Hemoglobin Pattern Distribution and TransfusionRequirements

 $26.3\pm5.1\%$ , p=0.42). Screening tests: The sickling test was positive in 85 cases (16.47%), of which 54% were confirmed to have hemoglobin variants on HPLC. The solubility test was positive in 82 cases (15.89%), with 56% confirmed as variants.

Caste	Frequency	Hb Variants found	Percent- age
Manelwarli	14	4	28.57%
Navhi	21	5	23.81%
Matang	9	2	22.22%
Banjara	26	5	19.23%
Mahar	22	4	18.18%
Others	424	50	11.79%
Total	516	70	13.57%



Variable	eta-Thal Major (n=7)	eta-Thal Trait (n=13)	Sickle Cell Dis- ease (n=17)	Sickle Cell Trait (n=29)	S-β Thal (n=2)
Hb (g/dL)	$\begin{array}{c} \textbf{4.97} \pm \\ \textbf{1.70} \end{array}$	$8.3\pm$ 1.47	7.8 ± 1.20	$\begin{array}{c}\textbf{9.81}\pm\\\textbf{1.14}\end{array}$	$\begin{array}{c}\textbf{8.3}\pm\\\textbf{0.14}\end{array}$
RBC (10 <sup>6</sup> /µL)	$\begin{array}{c}\textbf{3.68}\pm\\\textbf{1.41}\end{array}$	$\begin{array}{c} \textbf{4.1} \pm \\ \textbf{0.37} \end{array}$	3.95 ± 0.82	$\begin{array}{c} \textbf{4.25} \pm \\ \textbf{0.50} \end{array}$	$\begin{array}{c} \textbf{4.07} \pm \\ \textbf{0.99} \end{array}$
PCV (%)	15.54 土 3.27	26.28 ± 5.37	23.7 ± 3.28	28.7 ± 3.06	24.7 ± 0.42
MCV (fL)	70.5 ± 5.16	$\begin{array}{c} \textbf{73.30} \\ \pm \textbf{3.61} \end{array}$	72.7 ± 2.56	73.27 ± 3.06	67.85 ± 10.11
MCH (pg)	21.28 ± 1.25	21.14 ± 1.26	24.23 ± 3.08	23.69 ± 3.57	$\begin{array}{c} \textbf{20.6} \pm \\ \textbf{0.14} \end{array}$
MCHC (g/dL)	26.26 ± 7.63	21.89 ± 3.61	29.37 ± 1.24	30.41 ± 2.87	$\begin{array}{c} \textbf{25.4} \pm \\ \textbf{7.35} \end{array}$
HbA <sub>0</sub> (%)	10.35 ± 10.47	84.03 ± —	4.3 ± 2.58	51.85 ± 7.46	5.6 ± 2.12
HbA <sub>2</sub> (%)	2.07 ± 1.29	4.78 ± 0.87	3.35 ± 0.51	4.26 ± 0.96	$6.65\pm2.19$
HbF (%)	83.84 ± 12.67	0.87 ± 0.37	20.98 ± 1.85	$\begin{array}{c} \textbf{1.46} \pm \\ \textbf{1.47} \end{array}$	62.45 ± 5.44
HbS (%)	0	0	69.8 ± 9.82	34.49 ± 4.33	62.45 ± 5.44

Table 5: Hematological and Hemoglobin FractionationParameters Among Different Hemoglobinopathies

# **Mean with Standard Deviations**

The NESTROFT test was positive in 42 cases (8.14%), with a 52% confirmation rate on HPLC. (Table 6).

### DISCUSSION

Hemoglobinopathies are among the most common monogenic disorders worldwide, particularly prevalent in developing countries like India where consanguinity and endogamy contribute significantly to their inheritance patterns.

This study aimed to evaluate the prevalence and spectrum of hemoglobinopathies in anemic children using highperformance liquid chromatography (HPLC), and to correlate clinical, hematological, and biochemical findings with

Test Type	Partici- pants Tested	Positive Results	Vari- ants on HPLC	Sensi- tivity	Speci- ficity
Sickling Test	516	85 (16.47%)	46 (54%)	65.71%	91.26%
Solubility Test	516	82 (15.89%)	46 (56%)	65.71%	91.93%
NESTROFT Test	516	42 (8.14%)	22 (52%)	31.43%	95.52%

#### **Table 6: Screening Tests Compared to HPLC Results**

#### hemoglobin variant types.

In the present study, the prevalence of hemoglobinopathies was found to be 13.57% among anemic children, which is consistent with findings from other Indian studies, such as Shah et al.<sup>[15]</sup> (2012) who reported 13.1%, and Verma et al.<sup>[16]</sup> (2011) who found similar rates in pediatric populations using HPLC. This moderate-to-high prevalence reinforces the importance of routine screening for hemoglobin variants in symptomatic and anemic children.

Sickle cell trait (5.62%) was the most frequently detected abnormality, followed by sickle cell disease (3.29%), and  $\beta$ -thalassemia trait (2.52%). This aligns with data from western India, especially tribal and semi-urban areas of Maharashtra, where the sickle gene frequency is high. The findings are comparable to studies conducted by Madan et al. <sup>[17]</sup> (2010) and Patel et al. (2013) <sup>[18]</sup>, which reported similar variant distribution.

The majority of variant hemoglobin cases (70%) were observed in the 0.5–5-year age group, which may reflect both the early onset of symptoms and better detection due to increased awareness and healthcare-seeking behavior. This early detection is vital for timely initiation of interventions such as regular monitoring, prophylaxis for infections, and genetic counseling.

Consanguinity was reported in 30% of the variant hemoglobinopathy cases. This is a well-established risk factor for autosomal recessive disorders like  $\beta$ -thalassemia and sickle cell disease. Our findings are in concordance with those of Choudhary et al.<sup>[19]</sup> (2015), who also highlighted a strong link between parental consanguinity and the inheritance of hemoglobinopathies.

In terms of clinical presentation, pallor was the most common symptom both in the overall population and in the variant subgroup, followed by fatigue and breathlessness. These findings reflect the typical features of chronic anemia due to ineffective erythropoiesis and hemolysis. Fever and failure to thrive were also frequent, especially in children with sickle cell disease and thalassemia major, reflecting the systemic impact of these conditions.

The hematological profiles showed distinct patterns between normal and variant groups. Children with hemoglobinopathies had significantly lower hemoglobin (mean: 8.46 g/dL) and higher MCV and RDW values. The elevated MCV in variant cases may be due to compensatory erythropoiesis and bone marrow hyperplasia, especially in thalassemia carriers. RDW, a marker of anisopoikilocytosis, was significantly raised in variant cases (p=0.0226), reflecting ineffective erythropoiesis and red cell heterogeneity. These findings are in agreement with previous studies by Jain et al. <sup>[20]</sup> and Rangan et al. <sup>[21]</sup>

Out of 103 children who required blood transfusions, the highest requirement was noted in  $\beta$ -thalassemia major (71.4%) and sickle cell disease (41.2%). No transfusions were required in cases with  $\beta$ -thalassemia trait or HbE heterozygosity. This supports the classification of  $\beta$ -thalassemia major and sickle cell disease as clinically severe forms requiring intensive supportive care, while trait forms remain largely asymptomatic.

The caste-wise distribution revealed a higher frequency of hemoglobin variants in certain scheduled and tribal communities such as Manelwarli, Navhi, Matang, and Banjara. This likely reflects the genetic pooling due to sociocultural practices of endogamy.<sup>[22]</sup> These findings suggest that community-targeted screening programs may be highly effective in early identification and management.

HPLC proved to be a highly effective diagnostic tool, identifying abnormal hemoglobin fractions with precision. Among the screening tests, the sickling test, solubility test, and NESTROFT had moderate concordance with HPLC results, confirming variants in approximately 52–56% of cases. These conventional screening methods, although costeffective, have limited sensitivity and specificity, underscoring the importance of confirmatory testing with HPLC. <sup>[23]</sup>

This study also reported detailed fractionation profiles for each hemoglobinopathy. For instance, sickle cell disease patients had high HbS and HbF, while  $\beta$ -thalassemia trait was marked by elevated HbA2. Such detailed profiling is essential for distinguishing between similar clinical entities like SCD, SCT, and S- $\beta$  thalassemia.

Despite the strengths of this study, such as a sizeable sample and comprehensive analysis, limitations include its single-center design and exclusion of molecular confirmation techniques such as gene sequencing or ARMS-PCR. Additionally, neonatal cases and family studies were not included, which might have provided further insights into inheritance patterns.

#### CONCLUSIONS

This study highlights the significant burden of hemoglobinopathies among anemic children, with a prevalence rate of 13.57%, reinforcing the importance of early

detection and intervention. Sickle cell trait, sickle cell disease, and  $\beta$ -thalassemia trait emerged as the most common variants, with a notable prevalence particularly in younger children and specific caste groups linked to consanguinity. These findings underscore the genetic and regional factors driving these disorders, emphasizing their relevance as a public health concern in India.

High-performance liquid chromatography (HPLC) demonstrated its superiority over traditional screening methods for accurate diagnosis and classification, supporting its integration into clinical practice. The severity of conditions like  $\beta$ thalassemia major and sickle cell disease, marked by significant transfusion needs, further stresses the importance of timely management. Given these insights, communitybased screening, public education, and genetic counseling are essential strategies to reduce this preventable burden, particularly in high-risk populations. A comprehensive approach combining advanced diagnostics, clinical vigilance, and preventive measures is vital to address the challenge of hemoglobinopathies effectively.

### DISCLOSURE

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Conflict of Interest: None Declared

Author Contribution: All the authors involved in study have contributed equally at all stages of work

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