

Spectrum of hemoglobinopathies and hemoglobin variants detected by routine HPLC screening (high-performance liquid chromatography) in pediatric and obstetric population: a six-month tertiary hospital-based observational study

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Abstract

Background: Haemoglobinopathies are important inherited disorders with considerable clinical and genetic significance. High-performance liquid chromatography (HPLC) provides a rapid and reliable method for screening and characterisation of haemoglobin variants.

Objective: To determine the spectrum of haemoglobinopathies and haemoglobin variants detected by routine HPLC screening and to compare their

distribution according to age, sex and paediatric versus adult female/obstetric populations.

Materials and Methods: A hospital-based observational study was conducted at B.J. Wadia Hospital for Children, Parel, Mumbai, over six months from March 2026 to August 2026. A total of 1,881 HPLC records were analysed. Complete blood counts were performed using Mindray BC-6200/BC-7800 automated haematology analysers, and haemoglobinopathy screening was performed using the Bio-Rad D-10 Dual Program HPLC

system. Haemoglobin patterns were categorised according to HPLC findings. Categorical variables were expressed as frequencies and percentages, and associations were assessed using the Chi-square or Fisher's exact test, with $p < 0.05$ considered statistically significant.

Results: Among 1,881 cases, 1,714 (91.13%) showed no common abnormal haemoglobin pattern, while 157 (8.35%) had defined abnormal patterns. β -thalassaemia trait was the most common abnormality (72; 3.83%), followed by $\delta\beta$ -thalassaemia/HPFH (20; 1.06%), HbS homozygosity (15; 0.80%) and HbS heterozygosity (14; 0.74%). HbS homozygosity and HbS/ β -thalassaemia were predominantly observed in the paediatric population. Adult female/obstetric participants showed a higher proportion of normal patterns (94.6%) compared with children (63.0%). The overall difference in HPLC patterns between these groups was statistically significant ($\chi^2=376.21$, $p < 0.001$). Borderline HbA2 (3.6–3.9%) was identified in 10 cases (0.53%).

Conclusion: Routine HPLC screening demonstrated a diverse spectrum of haemoglobin abnormalities, with β -thalassaemia trait being the most common. Clinically significant sickling disorders were predominantly detected in children. Systematic HPLC screening with appropriate interpretation of borderline and abnormal patterns is valuable for early detection, counselling and further evaluation of haemoglobinopathies.

Keywords: Haemoglobinopathies, Haemoglobin Variants, High-Performance Liquid Chromatography (HPLC), B-Thalassaemia Trait, Sick-Cell Disease, Hbs; Hbe; Hbd Punjab

Introduction

Haemoglobinopathies are a heterogeneous group of inherited disorders caused by abnormalities in the

structure or synthesis of haemoglobin. They include thalassaemias and structural haemoglobin variants and constitute an important public health problem because affected individuals may range from clinically asymptomatic carriers to patients with severe chronic anaemia and other complications. In India, haemoglobinopathies are particularly important because of the considerable genetic, ethnic and geographical diversity of the population, resulting in marked regional variation in the distribution of different haemoglobin disorders.^{1,2}

β -thalassaemia and sickle-cell disorders are among the major haemoglobinopathies encountered in India. In addition, structural variants such as HbS, HbE and HbD Punjab occur with varying frequencies across different geographical regions and communities. A large multicentre Indian study involving 56,780 individuals reported an overall β -thalassaemia trait frequency of 2.78%, with substantial inter-regional variation. The same study demonstrated the presence of HbS, HbE, HbD and $\delta\beta$ -thalassaemia/HPFH patterns, highlighting the diversity of haemoglobin abnormalities in the Indian population.³

HbD Punjab is of particular relevance to the Indian subcontinent and is historically associated with the Punjab and north-western Indian region. Although HbD Punjab trait is generally clinically mild, its association with HbS can produce a clinically significant sickling disorder. Accurate identification of such compound patterns is therefore important during haemoglobinopathy screening.⁴

Early identification of haemoglobinopathies is important for clinical management, carrier detection, genetic counselling and prevention of severe inherited disorders. Screening of women during pregnancy or before

conception is particularly relevant because identification of a carrier can lead to testing of the partner and, when both partners are at risk, appropriate genetic counselling and prenatal diagnostic options. Indian guidelines recommend systematic screening and appropriate laboratory investigation of β -thalassaemia, sickle-cell disorders and other important haemoglobin variants^{2,5}.

High-performance liquid chromatography (HPLC) is an established laboratory technique for screening and characterising haemoglobinopathies. Cation-exchange HPLC allows separation and quantification of major haemoglobin fractions including HbA, HbA2 and HbF and facilitates detection of several common abnormal haemoglobins. Its advantages include automation, good resolution, reproducibility and quantitative assessment of haemoglobin fractions^{6,7}.

The importance of HPLC in routine haemoglobinopathy screening has also been demonstrated in large Indian laboratory-based studies. Warghade et al., in an analysis of 65,779 samples, reported β -thalassaemia trait, sickle-cell trait, sickle-cell disease, HbE trait and borderline HbA2 among the commonly detected abnormalities and concluded that cation-exchange HPLC was suitable for routine investigation because of its rapid analysis, resolution and quantitative capability⁸.

Interpretation of HPLC results nevertheless requires correlation with age, complete blood count findings and clinical information. Certain haemoglobin variants may show overlapping chromatographic characteristics, and borderline HbA2 values may create diagnostic difficulty in identifying β -thalassaemia carriers. Molecular studies from Indian referral centres have also demonstrated that some β -thalassaemia heterozygotes may have normal or borderline HbA2 levels, emphasising that HPLC findings should not always be interpreted in isolation^{7,9}.

Recent reviews have further emphasised the importance of combining appropriate screening methods with confirmatory investigations when required. HPLC is highly useful for initial screening and characterisation, but ambiguous or unusual patterns may require complementary techniques such as capillary electrophoresis or molecular analysis^{7,9}.

The present study was undertaken to evaluate the spectrum of haemoglobinopathies and haemoglobin variants detected by routine HPLC screening in a tertiary-care hospital. The study analysed HPLC patterns according to age and sex, compared haemoglobin profiles between paediatric and adult female/obstetric populations, and specifically assessed cases with borderline HbA2 levels. The study was designed to describe the pattern of haemoglobin abnormalities encountered in routine hospital-based screening and to highlight the importance of appropriate interpretation of HPLC findings in different patient populations.

Materials and Methods

Study Design and Setting

The present study was designed as a hospital-based observational study to evaluate the spectrum and distribution of hemoglobinopathies and hemoglobin variants detected by routine high-performance liquid chromatography (HPLC) screening. The study was conducted at B.J. Wadia Hospital for Children, Parel, Mumbai, over a period of six months from March 2026 to August 2026.

Study Population

The study included pediatric and adult individuals who underwent routine hemoglobinopathy screening by HPLC during the study period. A total of 1,881 HPLC screening records were included in the analysis. The study population comprised pediatric patients (<18 years), adult

female/obstetric cases (≥ 18 years), and adult male cases (≥ 18 years).

Inclusion Criteria

- Patients undergoing routine HPLC screening during the study period.
- Pediatric and adult patients with available HPLC results.
- Records with adequate demographic information for age and sex classification.

Exclusion Criteria

- Duplicate records.
- Records with inadequate or uninterpretable HPLC results.
- Records lacking essential demographic information required for analysis.

Sample Size and Study Duration

A total of 1,881 eligible HPLC screening records were analysed. The study duration was six months, from March 2026 to August 2026.

Sample Collection and Hematological Evaluation

Venous blood samples were collected in EDTA-containing anticoagulant tubes following standard laboratory procedures. Complete blood count (CBC) was performed as part of the routine hematological evaluation. Hematological parameters were analysed using Mindray BC-6200/BC-7800 automated hematology analysers. The CBC parameters included hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), total leukocyte count and platelet count, wherever available.

CBC findings were used as supportive hematological information during interpretation of HPLC patterns,

particularly in cases suggestive of thalassemia or other hemoglobinopathies.

HPLC Analysis

Hemoglobinopathy screening was performed using high-performance liquid chromatography (HPLC). The samples were analysed on the Bio-Rad D-10 Dual Program HPLC system according to the manufacturer's recommended operating procedures and the laboratory's established protocol.

The HPLC system separates hemoglobin fractions on the basis of their chromatographic properties and provides identification and quantification of major hemoglobin fractions and variants. The chromatographic patterns were interpreted according to the laboratory reporting criteria and established HPLC interpretation guidelines.

The following HPLC patterns were recorded and categorised:

1. Normal/no common abnormal hemoglobin pattern
2. β -thalassemia trait
3. $\delta\beta$ -thalassemia/HPFH pattern
4. HbS homozygous pattern
5. HbS heterozygous pattern
6. HbE heterozygous pattern
7. HbS/ β -thalassemia
8. HbE/ β -thalassemia
9. HbD Punjab heterozygous pattern
10. HbS + HbD Punjab
11. Borderline HbA2
12. Other/indeterminate patterns

Definition of Borderline HbA2

For the present study, borderline HbA2 was defined as an HbA2 level between 3.6% and 3.9%. These cases were analysed separately to determine their demographic distribution.

Age and Demographic Classification

Participants were classified into three major groups:

- **Pediatric:** <18 years
- **Adult female/obstetric:** ≥18 years
- **Adult male:** ≥18 years

For detailed age-wise analysis, the participants were further divided into ≤5 years, 6–10 years, 11–15 years, 16–18 years, 19–30 years, 31–40 years, 41–50 years, and >50 years.

Quality Control

Appropriate internal quality-control procedures were followed during hematological and HPLC analysis. Instrument performance was monitored using appropriate quality-control materials, and routine maintenance, calibration and quality-assurance procedures were performed according to laboratory protocols and manufacturer recommendations.

Data Collection

Relevant information was obtained from laboratory records and HPLC reports. The variables analysed included age, sex, demographic category and HPLC

Results and Observations

A total of 1,881 HPLC screening records were included in the present six-month observational study. The study population comprised pediatric and adult female/obstetric screening cases, along with adult male cases. HPLC patterns were categorised according to the interpretation provided in the laboratory reports.

Table 1: Overall distribution of HPLC patterns

HPLC pattern	No. of cases (n)	Percentage (%)
Normal / No common abnormal haemoglobin	1714	91.13
β-thalassemia trait	72	3.83
δβ-thalassemia / HPFH pattern	20	1.06
HbS homozygous	15	0.80
HbS heterozygous	14	0.74
HbE heterozygous	11	0.58
Borderline HbA2	10	0.53
HbS/β-thalassemia	7	0.37

pattern. The frequency and percentage of each hemoglobinopathy and hemoglobin variant were calculated. The distribution of abnormal HPLC patterns was further assessed according to age and sex.

Statistical Analysis

Categorical variables were expressed as frequency and percentage. The distribution of HPLC patterns between pediatric and adult female/obstetric populations was compared using the Chi-square test. Fisher's exact test was used where appropriate for small cell frequencies. A p-value <0.05 was considered statistically significant.

Age-wise and sex-wise distributions of individual hemoglobinopathy patterns were also analysed descriptively.

Ethical Considerations

Patient confidentiality was maintained throughout the study. Data were analysed in a manner that did not reveal individual patient identity, and the information obtained from routine laboratory records was used solely for research purposes.

HbD Punjab heterozygous	5	0.27
HbE/ β -thalassemia	2	0.11
HbS + HbD Punjab	1	0.05
Other/indeterminate patterns	10	0.53
Total	1881	100.00

Observation

The majority of screened individuals, 1,714 (91.13%), showed no common abnormal haemoglobin pattern on HPLC. Among the defined abnormal patterns, β -thalassemia trait was the most frequent finding (72, 3.83%), followed by $\delta\beta$ -thalassemia/HPFH-like patterns (20, 1.06%), HbS homozygous pattern (15, 0.80%) and HbS heterozygous pattern (14, 0.74%).

Overall, 157 cases (8.35%) showed a defined abnormal haemoglobin pattern, while an additional 10 cases (0.53%) were categorised as other/indeterminate. Ten cases (0.53%) demonstrated borderline HbA2 findings.

Table 2: Demographic distribution of the study population

Demographic characteristic	No. of cases (n)	Percentage (%)
Pediatric (<18 years)	165	8.77
Adult female/obstetric (≥ 18 years)	1593	84.69
Adult male (≥ 18 years)	123	6.54
Total	1881	100.00
Female	1668	88.68
Male	213	11.32

Observation

The study population was predominantly composed of adult female/obstetric screening cases (84.69%), followed by pediatric cases (8.77%) and adult male cases (6.54%). Overall, females constituted 88.68% of the study population, whereas males constituted 11.32%.

Pediatric cases were defined as patients aged <18 years. Adult females were considered the obstetric/adult female comparison group for the pediatric-versus-obstetric analysis.

Table 3: Comparison of HPLC patterns between pediatric and obstetric/adult female population

HPLC pattern	Pediatric n (%)	Obstetric/adult female n (%)	p-value
Normal / no common abnormal Hb	104 (63.0)	1507 (94.6)	<0.001
β -thalassemia trait	10 (6.1)	52 (3.3)	0.064
$\delta\beta$ -thalassemia/HPFH	14 (8.5)	6 (0.4)	<0.001
HbS homozygous	15 (9.1)	0 (0.0)	<0.001
HbS heterozygous	4 (2.4)	9 (0.6)	0.027
HbS/ β -thalassemia	6 (3.6)	0 (0.0)	<0.001

HbE heterozygous	2 (1.2)	6 (0.4)	0.169
HbE/ β -thalassemia	2 (1.2)	0 (0.0)	0.009
HbD Punjab heterozygous	0 (0.0)	4 (0.3)	1.000
HbS + HbD Punjab	1 (0.6)	0 (0.0)	0.094
Borderline HbA2	2 (1.2)	6 (0.4)	0.169
Other/indeterminate	4 (2.4)	3 (0.2)	<0.001

Overall comparison: $\chi^2 = 376.21$, $p < 0.001$.

Observation

A statistically significant difference was observed in the distribution of HPLC patterns between pediatric and obstetric/adult female populations ($p < 0.001$).

Normal HPLC patterns were significantly more frequent among obstetric/adult female cases (94.6%) compared with pediatric cases (63.0%).

Among pediatric patients, HbS homozygous (9.1%), $\delta\beta$ -thalassemia/HPFH (8.5%), β -thalassemia trait (6.1%) and HbS/ β -thalassemia (3.6%) were prominent abnormal patterns. In comparison, the obstetric/adult female population showed a predominance of β -thalassemia trait (3.3%), followed by HbS heterozygosity (0.6%) and HbE heterozygosity (0.4%).

Table 4: Age-wise distribution of hemoglobinopathy patterns

HPLC pattern	≤ 5 yr	6–10 yr	11–15 yr	16–18 yr	19–30 yr	31–40 yr	41–50 yr	>50 yr
β -thalassemia trait	4	4	2	1	36	20	3	2
$\delta\beta$ -thalassemia/HPFH	11	2	1	0	4	2	0	0
HbS homozygous	4	4	4	3	0	0	0	0
HbS heterozygous	2	0	2	0	5	4	1	0
HbS/ β -thalassemia	0	2	2	2	0	0	0	0
HbE heterozygous	2	0	0	0	5	4	0	0
HbE/ β -thalassemia	1	1	0	0	0	0	0	0
HbD Punjab heterozygous	0	0	0	0	3	2	0	0
HbS + HbD Punjab	0	1	0	0	0	0	0	0
Borderline HbA2	1	1	0	0	2	6	0	0
Other/indeterminate	4	0	1	0	2	2	0	0

Observation

Age-wise analysis demonstrated a clear concentration of clinically significant sickling patterns in childhood. All 15 cases of HbS homozygous pattern occurred below 18 years of age, while HbS/ β -thalassemia was also confined predominantly to pediatric patients.

The $\delta\beta$ -thalassemia/HPFH pattern was particularly frequent in younger children, with 11 cases in the ≤ 5 -year age group. In contrast, β -thalassemia trait was predominantly identified in adults, particularly those aged 19–30 years (36 cases) and 31–40 years (20 cases).

HbE and HbD Punjab heterozygous patterns were also predominantly detected in the adult age groups.

Table 5: Sex-wise distribution of major abnormal HPLC patterns

HPLC pattern	Male n (%)	Female n (%)
β -thalassemia trait	15	57
$\delta\beta$ -thalassemia/HPFH	6	14
HbS homozygous	12	3
HbS heterozygous	4	10
HbS/ β -thalassemia	5	2
HbE heterozygous	5	6
HbE/ β -thalassemia	2	0
HbD Punjab heterozygous	1	4
HbS + HbD Punjab	1	0
Borderline HbA2	2	8
Other/indeterminate	2	5

Observation

A female predominance was observed for most hemoglobinopathy patterns, particularly β -thalassemia trait (57 females) and borderline HbA2 (8 females), which corresponds with the predominance of adult female/obstetric screening in the study population.

In contrast, HbS homozygous and HbS/ β -thalassemia patterns showed a relative male predominance, with 12 of 15 HbS homozygous cases and 5 of 7 HbS/ β -thalassemia cases occurring among males.

Table 6: Borderline HbA2 (3.6–3.9%) cases

Parameter	Finding
Total borderline HbA2 cases	10
Pediatric cases	2
Adult female/obstetric cases	6
Adult male cases	2
≤ 5 years	1
6–10 years	1
19–30 years	2
31–40 years	6

Discussion

The present six-month hospital-based observational study evaluated 1,881 HPLC records from B.J. Wadia Hospital for Children, Parel, Mumbai, to determine the spectrum

of haemoglobinopathies and haemoglobin variants detected during routine screening. The study population was predominantly composed of adult females/obstetric participants, with smaller proportions of paediatric and

adult male participants. Overall, 1,714 cases (91.13%) showed no common abnormal haemoglobin pattern, while 157 cases (8.35%) demonstrated defined abnormal haemoglobin patterns. A further 10 cases (0.53%) were classified as other or indeterminate patterns.

β -thalassaemia trait was the most frequently detected defined abnormality in the present study, occurring in 72 cases (3.83%). This finding is consistent with the recognised importance of β -thalassaemia carrier states in India. In the large multicentre study by Mohanty et al., involving 56,780 individuals from six Indian cities, the overall prevalence of β -thalassaemia trait was 2.78%, although substantial regional and ethnic variation was observed [3]. The slightly higher frequency observed in the present hospital-based study may be related to the tertiary-care referral population and the indications for HPLC testing rather than representing population prevalence.

The predominance of β -thalassaemia trait in the present study also supports the continuing importance of carrier detection in routine haemoglobinopathy screening. Increased HbA2 is an important laboratory marker of β -thalassaemia trait, and HPLC provides a convenient method for its quantitative assessment^{6,7}. However, β -thalassaemia cannot be excluded solely on the basis of a normal or borderline HbA2 value because molecularly confirmed β -thalassaemia heterozygotes with normal or borderline HbA2 have been described in Indian referral populations⁹.

The $\delta\beta$ -thalassaemia/HPFH pattern was the second most frequent defined abnormality, occurring in 20 cases (1.06%). An important feature in the present study was its concentration in younger children, with 11 of the 20 cases occurring in the ≤ 5 -year age group. The interpretation of increased HbF requires careful consideration of age and

the complete haemoglobin profile. Indian multicentre and referral-centre studies have documented $\delta\beta$ -thalassaemia and HPFH among the spectrum of haemoglobin abnormalities encountered during screening^{3,9}.

HbS-related abnormalities represented another important component of the present study. HbS homozygosity was identified in 15 cases (0.80%), and all these cases occurred in the paediatric population. HbS/ β -thalassaemia was identified in seven cases (0.37%), with six occurring among children. This age distribution is clinically relevant because sickle-cell disease and compound sickling disorders may result in significant morbidity and require early diagnosis and appropriate follow-up. The World Health Organization emphasises that early diagnosis of sickle-cell disease is important for preventing complications and improving clinical management¹⁰.

HbS heterozygosity was detected in 14 cases (0.74%). Unlike HbS homozygosity, HbS heterozygosity occurred in both paediatric and adult populations. Recognition of sickle-cell trait is important not only for individual counselling but also for reproductive risk assessment because carriers can transmit the HbS allele to their offspring. Indian haemoglobinopathy guidelines therefore emphasise screening of partners of individuals carrying clinically important haemoglobin variants².

HbE heterozygosity was identified in 11 cases (0.58%), while HbE/ β -thalassaemia was identified in two cases (0.11%). HbE is an important haemoglobin variant in India, particularly in eastern and north-eastern populations. The multicentre study by Mohanty et al. demonstrated marked regional variation, with particularly high HbE trait frequencies in Assam and West Bengal³. The lower frequency observed in the present study is

therefore not unexpected given the hospital population and geographical setting.

HbD Punjab heterozygosity was identified in five cases (0.27%), while one case showed a combined HbS + HbD Punjab pattern. HbD Punjab is a structurally abnormal haemoglobin associated particularly with the Punjab and north-western Indian region. Although the heterozygous state is generally clinically mild, HbD Punjab in combination with HbS may produce a clinically significant sickling phenotype. Recognition of this combination is therefore important during HPLC interpretation⁴.

The paediatric and adult female/obstetric groups demonstrated a statistically significant difference in the distribution of HPLC patterns ($\chi^2 = 376.21$, $p < 0.001$). Normal haemoglobin patterns were considerably more common among adult females/obstetric participants than among paediatric participants (94.6% versus 63.0%). Conversely, several important abnormal patterns were disproportionately represented among children, including HbS homozygosity, HbS/ β -thalassaemia and $\delta\beta$ -thalassaemia/HPFH. This difference is likely influenced by the clinical indications for referral and the nature of screening in the two populations.

The high frequency of normal profiles among adult females/obstetric participants may reflect the use of HPLC primarily for antenatal or carrier screening. In contrast, paediatric referrals are more likely to be generated by clinical suspicion, family history, anaemia, abnormal red-cell indices or suspected haemoglobinopathy. Therefore, the observed difference between the two groups should not be interpreted as a population-level difference in disease prevalence.

Age-wise analysis demonstrated distinct patterns for different haemoglobin abnormalities. β -thalassaemia trait

was predominantly identified among adults, particularly in the 19–30-year and 31–40-year age groups. In contrast, all 15 HbS homozygous cases occurred below 18 years, while most HbS/ β -thalassaemia cases were also paediatric. The $\delta\beta$ -thalassaemia/HPFH pattern was particularly concentrated in children aged ≤ 5 years. These findings demonstrate the value of age-specific analysis when interpreting haemoglobinopathy screening data.

The sex-wise distribution showed female predominance for most abnormalities; however, this finding must be interpreted in the context of the marked female predominance of the overall study population. Females accounted for 1,668 of the 1,881 records (88.68%), whereas males accounted for only 213 (11.32%). Therefore, the higher absolute number of abnormal patterns among females does not necessarily indicate a biological female predisposition. The relative male predominance observed for HbS homozygosity and HbS/ β -thalassaemia should similarly be interpreted cautiously because of the unequal sex distribution.

Borderline HbA2 values between 3.6% and 3.9% were identified in 10 cases (0.53%). Two cases were paediatric, six were adult females/obstetric participants and two were adult males. Six of the 10 cases belonged to the 31–40-year age group. Borderline HbA2 values are clinically important because they may occur in individuals who require further evaluation for possible β -thalassaemia trait. Large Indian HPLC datasets have also reported borderline HbA2 as a recognised category during routine screening⁸.

Interpretation of borderline HbA2 results should preferably be combined with red-cell indices, clinical and family history, iron status and, when indicated, molecular analysis. In the present study, iron studies were not available; therefore, no conclusion regarding the

relationship between borderline HbA2 and iron deficiency could be made. Previous studies have shown that haemoglobinopathy diagnosis may become difficult when HbA2 values are close to the diagnostic threshold, and molecular studies have demonstrated β -thalassaemia mutations in some individuals with normal or borderline HbA2^{7,9}.

The present findings also reinforce the utility of HPLC as a routine screening technique. HPLC offers automated analysis, good resolution and quantitative estimation of haemoglobin fractions and can identify several common haemoglobin variants in a single analytical procedure [6–9]. However, HPLC should not be regarded as a substitute for confirmatory testing in every unusual or ambiguous case. Some variants may have overlapping retention characteristics, and molecular confirmation may be necessary for definitive characterisation of uncommon haemoglobin variants^{7,9}.

An important strength of the present study is the relatively large number of HPLC records analysed over a defined six-month period. The study also provides a detailed description of haemoglobin patterns according to age, sex and major patient groups and specifically evaluates borderline HbA2 results. The inclusion of both paediatric and adult female/obstetric populations allows comparison of the spectrum of haemoglobin abnormalities encountered in different clinical screening settings.

The study has certain limitations. It was conducted at a single tertiary-care hospital and therefore the findings cannot be considered representative of the general population. The study population was also markedly female predominant because of the large adult female/obstetric component. Referral and selection bias may consequently have influenced the observed frequencies. In addition, molecular confirmation was not

available for all abnormal HPLC patterns. Iron studies were also unavailable for the borderline HbA2 group, limiting further evaluation of these cases.

Despite these limitations, the present study demonstrates that routine HPLC screening can identify a broad spectrum of haemoglobin abnormalities, with β -thalassaemia trait being the most common defined abnormality. Clinically important sickling disorders and compound haemoglobinopathies were predominantly observed in the paediatric population, whereas β -thalassaemia trait and several structural variants were also identified among adults. The findings highlight the importance of systematic HPLC screening, age-appropriate interpretation, careful evaluation of borderline HbA2 results and confirmatory testing of ambiguous patterns.

Conclusion

Routine HPLC screening identified a diverse spectrum of haemoglobinopathies among the 1,881 cases studied. β -thalassaemia trait was the most common abnormality, while clinically significant sickling disorders were predominantly detected in children. The significant variation in haemoglobin patterns across age groups highlights the importance of systematic HPLC screening, appropriate interpretation of borderline HbA2 values, and confirmatory testing of ambiguous cases for effective haemoglobinopathy detection and counselling.

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