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SPECTRUM AND HAEMATOLOGICAL PROFILE OF HAEMOGLOBINOPATHIES AMONG CLINICALLY SUSPECTED PATIENTS AT A TERTIARY CARE CENTRE

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ABSTRACT

Background: Haemoglobinopathies show marked geographical and ethnic heterogeneity in India. Characterizing their regional spectrum together with red-cell indices and haemoglobin fractions can facilitate diagnosis, genetic counselling and targeted screening. This study evaluated the spectrum and haematological profiles of haemoglobinopathies among clinically suspected patients attending a tertiary referral centre in central India.

Materials and Methods: This prospective observational study included Paediatric and adult patients with microcytosis, hypochromia, sickling evidence or other clinical suspicion were evaluated. Complete blood counts, Leishman-stained peripheral smears, sickling-solubility testing, alkaline agarose-gel electrophoresis and Bio-Rad Variant II CE-HPLC were performed. Haemoglobin phenotypes were assigned by integrating clinical and laboratory findings. Frequencies, percentages and phenotype-specific haematological parameters were analysed.

Results: Of 805 evaluated patients, seven were excluded following recent transfusion and 798 were analysed. Haemoglobinopathies were identified in 635 participants, giving a diagnostic yield of 79.6% (95% CI: 76.6–82.2%). HbAS was the predominant phenotype (76.2%), followed by HbSS (15.9%), β-thalassaemia trait (2.5%), HbAE (1.7%) and HbJ Meerut (0.9%). Most affected participants were aged 11–20 years (33.2%), and age distribution differed significantly between HbAS and HbSS (p=0.036). Among HbSS patients, 43.6% had haemoglobin concentrations of 8–10 g/dL, 84.2% had RDW >15%, and mean HbF and HbS were 19.0% and 71.2%, respectively. β-Thalassaemia trait showed mean haemoglobin of 11.0 g/dL, MCV of 66.2 fL, RBC count of 5.28 million/mm³ and HbA_2 of 4.96%. CE-HPLC also detected HbS/β-thalassaemia, β-thalassaemia major/intermedia, HbEE, HbS/HbE, HbD, Hb Lepore and HPFH patterns.

Conclusion: The referral cohort demonstrated a predominantly sickle haemoglobinopathy spectrum. Combining CE-HPLC with haematological indices, smear morphology and clinical or family information enabled recognition of common and uncommon phenotypes. Rare or discordant patterns require confirmatory molecular investigation.

Keywords: cation-exchange HPLC; haematological profile; haemoglobin variants; haemoglobinopathies; sickle-cell disease; β-thalassaemia.

INTRODUCTION

Haemoglobinopathies comprise inherited quantitative defects of globin-chain synthesis, principally thalassaemias, and structural variants such as haemoglobins S, E and D. They are among the most prevalent monogenic disorders: approximately 7% of the global population carries a

haemoglobin variant, while β-thalassaemia affects about 40,000 newborns annually.[1] The Global Burden of Disease Study estimated that 7.74 million people were living with sickle-cell disease in 2021, with approximately 515,000 affected births and 376,000 associated deaths.[2] Early recognition is therefore important for treatment, genetic counselling and prevention of severe compound states.

India has a heterogeneous distribution of haemoglobin disorders because of population diversity, endogamy, migration and malaria-related selection. A large Indian series found HbS trait to be the most frequent variant, particularly among samples from Chhattisgarh.[3] At a western-Indian referral centre, 14,423 of 31,075 individuals had haemoglobin abnormalities, encompassing 49 β-thalassaemia mutations and several uncommon variants.[4] Regional detection rates differ substantially: 50.2% among anaemic tertiary-care patients in Odisha.[5] 11.26% in a Saurashtra population-screening cohort.[6] 20.12% among anaemic patients in western India.[7] and 27.9% among clinically selected patients in southern India.[8] Antenatal screening across five states recently identified carrier states in 6.8% of 33,270 women.[9] Hospital detection rates must therefore not be interpreted as community prevalence.

Clinical expression ranges from asymptomatic carriage to haemolytic anaemia, jaundice, splenomegaly, vaso-occlusive illness and transfusion dependence. Haematological parameters provide essential diagnostic context: β-thalassaemia trait usually produces disproportionate microcytosis and hypochromia with a relatively preserved erythrocyte count, whereas sickle-cell disease is associated with anaemia, reticulocytosis and increased red-cell distribution width. Recent CE-HPLC studies demonstrate phenotype-related differences in haemoglobin, MCV, MCH, erythrocyte count, RDW and HbF.[10] However, integrated data describing the spectrum and haematological profiles of clinically suspected patients in India remain limited. This study therefore aimed to enumerate haemoglobinopathies and characterize their demographic and haematological patterns at a tertiary referral centre.

MATERIALS AND METHODS

Study design and setting: This prospective hospital-based observational study was conducted from June 2012 to May 2015 in the Department of Pathology, of a tertiary care centre, in Central India. The study aimed to determine the spectrum of haemoglobinopathies among clinically suspected patients and describe their demographic, haematological and haemoglobin-fraction profiles.

Study population and eligibility

Paediatric and adult patients referred for haemoglobinopathy evaluation were eligible when they had one or more of the following: mean corpuscular volume (MCV) ≤80 fL, mean corpuscular haemoglobin (MCH) <27 pg, sickled erythrocytes on peripheral smear, a positive sickling-solubility test, or clinical suspicion of a haemoglobin disorder. Available family members were also evaluated when clinically indicated. Neonates and patients reporting a blood transfusion during the preceding four months were excluded because transfused erythrocytes could alter haemoglobin fractions. Written informed consent was obtained after explaining the procedure. During the study period, 805 patients were enrolled; seven were excluded for recent transfusion, leaving 798 for analysis.

Clinical assessment and specimen processing: Demographic details, presenting manifestations, relevant medical and transfusion history, and family history were recorded using a structured proforma. Under aseptic precautions, 3 mL of venous blood was collected into a dipotassium ethylenediaminetetraacetic acid (K_2EDTA) tube. Samples not analysed immediately were refrigerated at 2–8°C and tested within seven days. Complete blood counts were generated using a Beckman Coulter HMX five-part differential analyser. Haemoglobin concentration, erythrocyte count, MCV, MCH, mean corpuscular haemoglobin concentration and red-cell distribution width were recorded. Leishman-stained peripheral smears were examined for red-cell morphology, including microcytosis, hypochromia, anisopoikilocytosis, target cells, sickled cells, polychromasia and nucleated erythrocytes. Sickling-solubility testing was performed in relevant cases, while brilliant cresyl blue staining for HbH inclusions was undertaken when indicated.

CE-HPLC analysis: All specimens were analysed using the Bio-Rad Variant II cation-exchange high-performance liquid chromatography system with the β-Thalassaemia Short Program. For each analysis, 5 µL of EDTA-anticoagulated blood was diluted in 1 mL of the manufacturer's haemolysate reagent. Haemoglobin fractions were separated using a phosphate-buffer gradient and detected at 415 nm, with 690 nm used for background correction. The system generated a chromatogram in approximately 6.5 minutes and reported retention time, peak area and percentage. Manufacturer-defined windows were P1 (0.63–0.85 minutes), HbF (0.98–1.20), P2 (1.24–1.40), P3 (1.40–1.90), HbA_0 (1.90–3.10), HbA_2/E (3.30–3.90), HbD (3.90–4.30), HbS (4.30–4.70) and HbC (4.90–5.30).

Electrophoresis and phenotype assignment: Alkaline agarose-gel electrophoresis was performed for every sample using the Maestro 101 system at pH 8.4–8.6. Washed erythrocytes were lysed, 3 µL of haemolysate was applied, and electrophoresis was conducted at 150 V for 30 minutes. Gels were fixed, stained with Amido Black, destained, dried and quantified by TurboScan densitometry. Phenotypes were assigned by integrating clinical information, red-cell indices, smear findings, sickling results, electrophoretic mobility and HPLC retention

characteristics. HbAS was defined by HbS 21–45% with HbS below HbA; HbSS by HbS >45% and exceeding HbA; β-thalassaemia trait by HbA_2 4–9%; and HbS/β-thalassaemia by raised HbA_2 with HbS >45%. HbE, HbD, HbJ Meerut, Hb Lepore and hereditary persistence of fetal haemoglobin were identified from their characteristic quantitative, retention and migration patterns. Discordant findings were reviewed using all available evidence.

Outcome measures: The primary outcome was the frequency and proportional distribution of individual haemoglobinopathy phenotypes. Secondary outcomes included age distribution and phenotype-specific haematological profiles.

Statistical analysis: Analyses were performed using SPSS 21.0. Categorical variables were summarized as frequencies and percentages, and continuous variables as mean±standard deviation. The chi-square test was used for categorical comparisons; continuous variables were compared using the unpaired Student's t-test or analysis of variance, as appropriate. Pearson's correlation assessed relationships between continuous measurements. Tests were two-sided, with $p<0.05$ considered statistically significant.

RESULTS

A total of 805 clinically suspected patients were evaluated. Seven (0.9%) were excluded because of blood transfusion during the preceding four months, leaving 798 participants for analysis. An abnormal haemoglobin pattern was detected in 635 participants, giving a diagnostic yield of 79.6% (95% CI: 76.6–82.2%), while 163 (20.4%) had no detectable abnormal haemoglobin [Table 1].

Table 1: Participant recruitment and diagnostic yield among clinically suspected patients.

Study stage or diagnostic outcome	Frequency	%	Denominator
Patients initially evaluated	805	100	805
Excluded because of transfusion during preceding four months	7	0.9	805
Patients included in final analysis	798	99.1	805
Abnormal haemoglobin pattern detected	635	79.6	798
No abnormal haemoglobin detected	163	20.4	798

The abnormal-pattern detection rate was 79.6% (95% CI: 76.6–82.2%). This represents diagnostic yield in a clinically selected referral population and should not be interpreted as community prevalence. CI, confidence interval.

Sickle-cell trait was the predominant phenotype, accounting for 484/635 cases (76.2%), followed by sickle-cell disease in 101 (15.9%). β-Thalassaemia trait was identified in 16 (2.5%), haemoglobin E trait in 11 (1.7%), and haemoglobin J Meerut trait in six (0.9%). Less frequent abnormalities included HbS/β-thalassaemia in four (0.6%), HbS/HbE in three (0.5%), β-thalassaemia major/intermedia, HbEE, HbDD and HPFH in two cases each (0.3%), and HbAD and Hb Lepore in one case each (0.2%) [Table 2].

Table 2: Spectrum of haemoglobinopathies among patients with abnormal haemoglobin patterns.

Haemoglobinopathy phenotype	Frequency	%
Sickle-cell trait (HbAS)	484	76.2
Sickle-cell disease (HbSS)	101	15.9
β-Thalassaemia trait	16	2.5
β-Thalassaemia major/intermedia	2	0.3
HbS/β-thalassaemia	4	0.6
Haemoglobin E trait (HbAE)	11	1.7
Homozygous haemoglobin E (HbEE)	2	0.3
Compound heterozygous HbS/HbE	3	0.5
Haemoglobin J Meerut trait	6	0.9
Haemoglobin D trait (HbAD)	1	0.2
Homozygous haemoglobin D (HbDD)	2	0.3
Haemoglobin Lepore	1	0.2
Hereditary persistence of fetal haemoglobin	2	0.3
Total	635	100

Percentages may not total exactly 100 because of rounding. Hb, haemoglobin; HPFH, hereditary persistence of fetal haemoglobin. No p value was calculated because this table presents the descriptive distribution of mutually exclusive phenotypes.

The highest proportion of haemoglobinopathies occurred in participants aged 11–20 years (211/635; 33.2%), followed by those aged 21–30 years (145; 22.8%) and 31–40 years (98; 15.4%). Among HbAS cases with documented age, 34.2% were aged 11–20 years, whereas 39.6% of HbSS cases occurred in this age group. The overall age distribution differed significantly between HbAS and HbSS phenotypes ($\chi^2=10.29$; df=4; $p=0.036$) [Table 3].

Table 3: Age distribution of haemoglobinopathy cases and comparison between HbAS and HbSS.

Age group	All haemoglobinopathies (N=635)	HbAS with documented age (n=480)	HbSS (n=101)
	Frequency (%)		
0–10 years	87 (13.7)	67 (14.0)	9 (8.9)
11–20 years	211 (33.2)	164 (34.2)	40 (39.6)
21–30 years	145 (22.8)	100 (20.8)	32 (31.7)

31–40 years	98 (15.4)	77 (16.0)	10 (9.9)
≥41 years	94 (14.8)	72 (15.0)	10 (9.9)
Total with documented age	635 (100.0)	480 (100.0)	101 (100.0)
Overall comparison	χ^2=10.29; df=4, p=0.036		

The p value compares the age distribution of HbAS and HbSS cases using the chi-square test. Four of the 484 HbAS cases lacked placement in the phenotype-specific age table and were excluded from this comparison. The overall age table contained data for all 635 affected participants. df, degrees of freedom; HbAS, sickle-cell trait; HbSS, sickle-cell disease.

Among 101 HbSS patients, the most frequent haemoglobin concentration was 8–10 g/dL, observed in 44 (43.6%), while 24 (23.8%) had haemoglobin below 8 g/dL. MCV was 73–100 fL in 70 (69.3%), and 22 (21.8%) demonstrated macrocytosis. Normal-range MCHC values of 31–37 g/dL were present in 90 (89.1%). RDW was 15–25% in 72 (71.3%), while 13 (12.9%) had RDW >25%, with corresponding mean HbS values of 71.3% and 75.6%. HbF was ≥10% in 84 (83.2%) patients, including seven (6.9%) with HbF >30% [Table 4].

Table 4: Haematological profile of patients with homozygous sickle-cell disease.

Parameter	Category	Frequency (%)	Mean HbS (%)
Haemoglobin concentration	<5 g/dL	1 (1.0)	—
	5–7.9 g/dL	23 (22.8)	—
	8–10 g/dL	44 (43.6)	—
	>10 g/dL	33 (32.7)	—
MCV	<72 fL	9 (8.9)	—
	73–100 fL	70 (69.3)	—
	>100 fL	22 (21.8)	—
MCH	<27 pg	25 (24.8)	—
	27–32 pg	44 (43.6)	—
	>32 pg	32 (31.7)	—
MCHC	<31 g/dL	11 (10.9)	—
	31–37 g/dL	90 (89.1)	—
RDW	<15%	16 (15.8)	67.4
	15–25%	72 (71.3)	71.3
	>25%	13 (12.9)	75.6
HbF	<10%	17 (16.8)	71.2
	10–19.9%	39 (38.6)	73.7
	20–30%	38 (37.6)	70.9
	>30%	7 (6.9)	67

N=101. Hb, haemoglobin; HbF, fetal haemoglobin; HbS, sickle haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red-cell distribution width. Inferential testing was not possible because individual observations or dispersion measures were unavailable.

HbS/β-thalassaemia cases had a mean haemoglobin concentration of 8.2 g/dL, MCV of 71.6 fL, MCH of 24.0 pg, RBC count of 3.36 million/mm³ and RDW of 23.3%. β-Thalassaemia-trait cases demonstrated lower MCV and MCH values (66.2 fL and 20.0 pg) but a higher mean RBC count of 5.28 million/mm³. The two β-thalassaemia major/intermedia cases had mean haemoglobin of 7.0 g/dL and markedly increased RDW of 36.6% [Table 5].

Table 5: Mean haematological parameters in HbS/β-thalassaemia and β-thalassaemia phenotypes.

Haematological parameter	HbS/β-thalassaemia (n=4)	β-Thalassaemia trait (n=16)	β-Thalassaemia major/intermedia (n=2)
Haemoglobin, g/dL	8.2	11	7
RBC count, million/mm³	3.36	5.28	3.01
MCV, fL	71.6	66.2	75
MCH, pg	24	20	23.1
MCHC, g/dL	33	31	30.5
RDW, %	23.3	16.5	36.6

MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell; RDW, red-cell distribution width. Statistical comparisons were not undertaken because standard deviations and participant-level observations were unavailable and two groups contained only four and two cases.

HbAS cases had a mean HbS fraction of 32.1% (range 21–45%), with mean HbA of 57.0%. In HbSS, mean HbS was 71.2% (range 46–93%) and mean HbF was 19.0%. HbS/β-thalassaemia demonstrated mean HbS, HbF and HbA_2 values of 66.0%, 22.0% and 5.40%, respectively. β-Thalassaemia trait was characterized by mean HbA_2 of 4.96% (range 4.2–6.0%), whereas β-thalassaemia major/intermedia demonstrated mean HbF of 34.3% [Table 6].

Table 6: CE-HPLC haemoglobin-fraction profile of the principal haemoglobinopathy phenotypes.

Phenotype	Frequency	HbA, mean %	HbA_2, mean %	HbF, mean %	HbS, mean %	Additional findings
HbAS	484	57	3.6	1.5	32.1	HbS range 21–45%
HbSS	101	7.8	3.5	19	71.2	HbS range 46–93%
HbS/β-thalassaemia	4	8.3	5.4	22	66	HbS range 62–69%; HbA_2 range 4.7–6.3%
β-Thalassaemia trait	16	83.4	4.96	0.8	—	HbA_2 range 4.2–6.0%
β-Thalassaemia major/intermedia	2	57	4.9	34.3	—	Both cases were transfusion-dependent on follow-up

CE-HPLC, cation-exchange high-performance liquid chromatography; HbA, adult haemoglobin; HbA_2, minor adult haemoglobin; HbF, fetal haemoglobin; HbS, sickle haemoglobin. Values are descriptive means. Statistical comparison across phenotypes was inappropriate because dispersion measures were unavailable and subgroup sizes were markedly unequal.

Among the less frequent phenotypes, HbE trait showed a mean HbA_2/E-window fraction of 26.6%, while the two HbEE cases had a mean HbE fraction of 84.2%. The three HbS/HbE cases demonstrated mean HbA, HbA_2/E and HbF fractions of 3.3%, 33.1% and 5.0%, respectively. Six HbJ Meerut cases had a mean P3-window fraction of 16.7% and mean retention time of 1.73 minutes. HbD fractions ranged from 33% to 87%, with retention times of 4.09–4.17 minutes. The two HPFH cases were asymptomatic and had mean HbF of 26.5% [Table 7].

Table 7. CE-HPLC characteristics of less frequently detected haemoglobinopathies.

Phenotype	Frequency	Principal CE-HPLC findings	Supporting findings
Haemoglobin E trait	11	Mean HbA_2/E-window fraction 26.6%; HbE below HbA	HbE co-eluted with HbA_2
Homozygous haemoglobin E	2	Mean HbE fraction 84.2%; HbE exceeded HbA	Homozygous pattern
Compound HbS/HbE	3	Mean HbA 3.3%, HbA_2/E 33.1% and HbF 5.0%	Family evaluation supported diagnosis in two cases
Haemoglobin J Meerut trait	6	Mean P3 fraction 16.7% (range 11–26%); mean retention time 1.73 minutes	Fast-moving electrophoretic band; mean HbA 74.3% and HbA_2 2.1%
Haemoglobin D trait	1	HbD <50% and below HbA	Peak in D window
Homozygous haemoglobin D	2	HbD >50% and exceeding HbA	HbD values across HbD cases ranged from 33–87%; retention time 4.09–4.17 minutes
Haemoglobin Lepore	1	Abnormal fraction within the HbA_2/E-related region	Required correlation with the full chromatographic and electrophoretic pattern
HPFH phenotype	2	Mean HbA 66.3%, HbA_2 2.4% and HbF 26.5%	Both participants were clinically asymptomatic

CE-HPLC, cation-exchange high-performance liquid chromatography; Hb, haemoglobin; HbF, fetal haemoglobin; HPFH, hereditary persistence of fetal haemoglobin. Because these phenotypes contained only one to six cases, values are descriptive and no p values were calculated.

DISCUSSION

The present study identified haemoglobinopathies in 635 of 798 evaluable clinically suspected patients, yielding 79.6% (95% CI: 76.6–82.2%). This figure is substantially higher than the 12.17% abnormality rate reported by Mondal et al., among 119,336 tertiary-care samples.[11] but resembles sickle-focused referral data from southern Odisha by Sahu et al., where 59.2% of 2,332 suspected patients had abnormal haemoglobin patterns.[12] A recent rural tertiary-centre study by Yadav et al., detected haemoglobinopathies in only 13.8% of 392 tested patients.[13] These differences primarily reflect case selection, referral pathways and regional genetic structure; the present cohort required microcytosis, hypochromia, sickling evidence or clinical suspicion and included some family members. Consequently, the observed yield quantifies diagnostic enrichment rather than population prevalence.

HbAS constituted 76.2% of abnormal cases and HbSS 15.9%, demonstrating a strongly sickle-predominant spectrum. In study by Upadhye et al., Central-Indian newborn screening similarly identified 978 HbAS and 104 HbSS infants among 2,134 screened newborns.[14] Jain et al., also showed that central-Indian sickle disease includes HbSS, HbS/β-thalassaemia and HbS/HbD-Punjab, with considerable clinical severity despite the Asian haplotype.[15] The four HbS/β-thalassaemia cases in the present series are biologically consistent with the documented β-thalassaemia mutation diversity across Chhattisgarh populations in study by Jha et al,[16] Most diagnoses occurred at 11–20 years, and the age distribution differed between HbAS and HbSS (χ^2=10.29, p=0.036). Earlier recognition of HbSS probably reflects symptomatic anaemia or vaso-occlusive manifestations, whereas asymptomatic carriers are commonly detected later through family, antenatal or preoperative testing.

Among HbSS patients, 43.6% had haemoglobin concentrations of 8–10 g/dL and 23.8% had values below 8 g/dL, consistent with chronic haemolysis and

shortened erythrocyte survival. Although 69.3% were normocytic, 21.8% had macrocytosis, which may reflect reticulocytosis, folate deficiency or treatment-related effects; treatment exposure was not documented. RDW exceeded 15% in 84.2%, and mean HbS increased from 67.4% in the lowest RDW category to 75.6% when RDW exceeded 25%, suggesting greater erythrocyte heterogeneity with higher sickle burden. Mean HbF was 19.0%, while 83.2% had HbF ≥10%. Jain et al., reported mean HbF of 24.4% in central-Indian HbSS.[15] Indian molecular studies by Hariharan et al., and Hariharan et al., further show that HMOX1, BCL11A, HBS1L-MYB and γ-globin variants contribute to HbF variability and milder haematological phenotypes.[17,18] HbF inhibits HbS polymerization by lowering the intracellular HbS concentration and disrupting polymer assembly. However, mean HbA of 7.8% in the HbSS group is unexpected following exclusion of recent transfusion and warrants verification of transfusion history, genotype and phenotype assignment.

The thalassaemic phenotypes displayed biologically coherent red-cell patterns. β-Thalassaemia trait showed marked microcytosis (MCV 66.2 fL), hypochromia (MCH 20.0 pg), a relatively preserved erythrocyte count (5.28 million/mm³) and mean HbA_2 of 4.96%. Reduced β-chain synthesis produces small, poorly haemoglobinized erythrocytes, while compensatory erythropoiesis maintains the cell count. Similar profiles have been documented in a large North-Indian series by Tripathi et al,.[19] and among Tharu adolescents in study by Nigam et al., where mean HbA_2 was significantly increased in β-thalassaemia trait.[20] Nevertheless, MCV and MCH alone are imperfect carrier-screening tools, as demonstrated in a diverse Himachal Pradesh population as found in study by Bharti et al,.[21] HbS/β-thalassaemia showed lower haemoglobin (8.2 g/dL), increased RDW (23.3%), HbS of 66.0%, HbA_2 of 5.4% and HbF of 22.0%, reflecting combined sickling, ineffective erythropoiesis and variable residual β-chain production. The two β-thalassaemia major/intermedia cases had haemoglobin of 7.0 g/dL, RDW of 36.6% and HbF of 34.3%, consistent with severe globin-chain imbalance.

The detection of HbE, HbD, HbJ Meerut, HbS/HbE, Hb Lepore and HPFH illustrates the molecular diversity encountered even in a sickle-predominant region. A recent Indian review by Thaker et al., catalogued 63 rare or novel haemoglobin variants, including HbJ Meerut.[22] Molecular evaluation of suspected δβ-thalassaemia/HPFH has also demonstrated substantial phenotypic overlap despite increased HbF in study by Gupta et al,[23] Although CE-HPLC enables rapid presumptive identification through retention windows and fraction percentages, recent prospective evidence in study by Anandani et al., emphasizes interpretative difficulties involving HbE, Hb Lepore, HbJ Meerut and increased HbF.[24] Therefore, rare, borderline or internally inconsistent profiles should be correlated with complete blood counts, smear morphology, family studies and confirmatory molecular testing.

Limitations: This single-centre study included clinically suspected patients and selected family members; consequently, its 79.6% diagnostic yield cannot be generalized as population prevalence. Several uncommon phenotypes contained very few cases, limiting statistical comparisons. Molecular confirmation was not performed universally, treatment and nutritional status were incompletely documented, and some source tables contained missing or internally inconsistent entries. The cross-sectional laboratory assessment also prevented evaluation of clinical outcomes or temporal changes.

CONCLUSION

The study demonstrates a predominantly sickle haemoglobinopathy spectrum among clinically suspected patients attending a tertiary referral centre in central India, with HbAS and HbSS accounting for most abnormalities. HbSS was characterized by moderate anaemia, increased RDW and substantial HbF expression, whereas β-thalassaemia trait showed marked microcytosis, hypochromia, a relatively preserved erythrocyte count and increased HbA_2. CE-HPLC additionally identified uncommon HbE, HbD, HbJ Meerut, Hb Lepore and HPFH patterns. Integrating haemoglobin fractions with complete blood counts, smear morphology, clinical history and family evaluation is essential for accurate classification. Borderline, rare or internally inconsistent patterns should undergo molecular confirmation before definitive counselling or treatment decisions.

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