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Integrated laboratory evaluation of hemoglobinopathies in an Indian tertiary care center: A hematological, HPLC, and molecular study

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Abstract

Background: Hemoglobinopathies are a significant diagnostic and preventive challenge in India, with thalassemias being the most common inherited disorders. Overlapping hematological indices and hemoglobin fraction patterns often complicate detection, particularly in carriers and compound heterozygotes. This study evaluates the diagnostic spectrum and utility of hematological parameters, HPLC, and molecular testing for accurate classification.

Materials and methods: This ambispective observational study included 282 patients evaluated for suspected hemoglobinopathies from June 2022 to June 2023. Retrospective data (June–December 2022) and prospective cases (January–June 2023) were analyzed using standardized protocols. Investigations included complete blood counts, red cell indices, reticulocyte counts, sickling and solubility tests, peripheral smear, and HPLC. Cases were categorized into thalassemia and sickle cell disorders. Molecular testing was performed in selected cases with inconclusive or borderline findings. Statistical analysis employed one-way ANOVA and chi-square tests.

Results: Thalassemias were the predominant disorders. β-thalassemia trait showed elevated RBC counts with low MCV and MCH and increased HbA2, while α-thalassemia presented with microcytosis and normal HbA2 and HbF, requiring molecular confirmation. Common β-thalassemia mutations included IVS-I-5 (G>C), Codon 41/42 (–CTTT), Codon 8/9 (+G), and 619 bp deletion; α-thalassemia was mainly associated with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions. Significant correlations were observed between hematological indices, HPLC findings, and molecular results ($p < 0.001$).

Conclusion: Thalassemias remain the most prevalent hemoglobinopathies in Indian tertiary care settings. An integrated approach combining hematological indices, HPLC, and targeted molecular testing is essential for accurate diagnosis, carrier detection, and genetic counseling.

Keywords: hemoglobinopathies; thalassemia; sickle cell disease; HPLC; molecular testing; carrier screening

Introduction

Hemoglobinopathies represent a heterogeneous group of inherited disorders affecting hemoglobin synthesis and structure and constitute a significant public health burden worldwide, particularly in developing countries [1]. In India, these disorders are among the most common monogenic diseases, with thalassemias and sickle cell disorders accounting for the majority of cases [2, 3]. The high carrier frequency, wide ethnic diversity, and regional clustering of mutations contribute to the substantial disease burden observed in tertiary care settings [4].

Thalassemias, resulting from reduced or absent synthesis of α- or β-globin chains, display a broad clinical and hematological spectrum ranging from asymptomatic carrier states to transfusion-dependent disease [1]. β-thalassemia trait is the most frequently

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encountered form in routine laboratory practice, while α-thalassemia often remains underdiagnosed due to subtle hematological changes and the absence of definitive abnormalities on conventional hemoglobin analysis [5]. The coexistence of multiple hemoglobin variants and compound heterozygous states further complicates accurate laboratory diagnosis [6].

Initial evaluation of suspected hemoglobinopathies relies on complete blood counts, red cell indices, and peripheral smear examination. High-performance liquid chromatography (HPLC) has emerged as a robust and widely used modality for hemoglobin variant analysis, allowing reliable quantification of HbA2 and HbF and identification of common hemoglobin variants [7, 8]. However, overlapping HPLC patterns, borderline HbA2 values, and normal chromatograms in α-thalassemia carriers pose diagnostic challenges, particularly in population-based screening programs [9].

Molecular characterization plays a pivotal role in resolving these diagnostic ambiguities by enabling precise identification of globin gene mutations prevalent in the Indian population [10]. Common β-thalassemia mutations such as IVS-I-5 (G>C), Codon 41/42 (–CTTT), Codon 8/9 (+G), and the 619 bp deletion, along with α-globin gene deletions like $-\alpha^{3.7}$ and $-\alpha^{4.2}$, account for a substantial proportion of cases. Accurate detection of carriers through integrated hematological, biochemical, and molecular approaches is central to effective prevention strategies, including genetic counselling and prenatal diagnosis [3, 4].

Despite national screening initiatives, there remains limited consolidated data from tertiary care centers on the laboratory diagnostic spectrum of hemoglobinopathies and the relative contribution of molecular testing in routine practice.

Therefore, the present study was undertaken to evaluate the spectrum of hemoglobinopathies diagnosed over a one-year period at a tertiary care center in India, to correlate hematological parameters with HPLC and molecular findings, and to emphasize the critical role of comprehensive laboratory evaluation in carrier detection and prevention of hemoglobinopathies [11].

Materials and methods

This ambispective observational study was conducted at a tertiary care center over a one-year period from June 2022 to June 2023. The retrospective component included data retrieved from laboratory records (June 2022–December 2022), while the prospective component (January 2023–June 2023) involved real-time evaluation using standardized diagnostic protocols to minimize inter-observer variability. All consecutive patients, irrespective of age or sex, who were evaluated for suspected hemoglobinopathies during the study period were included in the analysis. Patients with incomplete laboratory workup, inadequate sample volume, hemolyzed samples, or repeat evaluations of the same patient were excluded. No pre-specified sample size was calculated; instead, all eligible cases during the defined period were included to ensure comprehensive representation of the diagnostic spectrum. The study was approved by the Institutional Ethics Committee, and a waiver of informed consent was granted as the study involved retrospective analysis of anonymized laboratory data.

Demographic details including age and sex, along with relevant laboratory parameters, were retrieved from laboratory information system records. Hematological evaluation included complete blood counts with red cell indices (hemoglobin, RBC count, MCV, MCH, MCHC, and RDW), reticulocyte counts, and peripheral blood smear examination. Screening tests for sickling, including sickling test and solubility test, were performed where clinically indicated. Based on hematological findings and hemoglobin analysis, cases were categorized into β-thalassemia trait, β-thalassemia intermedia, β-thalassemia major, α-thalassemia, sickle cell trait, sickle cell disease, sickle–β-thalassemia, and other compound heterozygous hemoglobin variants.

Hemoglobin variant analysis was performed using high-performance liquid chromatography (HPLC) on an automated system, following the manufacturer's standard protocol. HbA2, HbF, and variant hemoglobin fractions were quantified and interpreted using established diagnostic cut-offs. Internal quality controls were run with each batch, and chromatograms were independently reviewed by two experienced hematopathologists to ensure diagnostic accuracy.

Molecular analysis was performed in selected cases based on predefined criteria, including inconclusive or borderline HPLC findings (e.g., HbA2 levels between 3.2–3.5%), suspected α-thalassemia with normal HPLC profiles, discrepancies between hematological indices and HPLC results, suspected compound heterozygous states such as sickle–β-thalassemia, and cases requiring confirmation for genetic counseling. Strip-based molecular assays were employed to detect common β-globin gene mutations prevalent in the Indian population, including IVS-I-5 (G>C), Codon 41/42 (–CTTT), Codon 8/9 (+G), and the 619 bp deletion. For α-thalassemia, deletional mutations such as $-\alpha^{3.7}$ and $-\alpha^{4.2}$ were assessed. Molecular results were interpreted in conjunction with hematological and HPLC findings.

Statistical analysis was performed using SPSS software version 26.0. Normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequencies and percentages. One-way analysis of variance (ANOVA) was used to compare hematological parameters across different diagnostic categories. For variables showing significant differences on ANOVA, post-hoc Tukey analysis was performed to identify intergroup differences. Chi-square tests was applied to assess associations between categorical variables. A p-value of <0.05 was considered statistically significant.

Results

A total of 282 patients were evaluated for suspected hemoglobinopathies during the study period. The cohort comprised 154 males (54.6%) and 128 females (45.4%), with a mean age of 21.8 ± 14.6 years (range: 6 months–65 years). Pediatric patients (<18 years) constituted 44.3% of the study population. All cases had complete hematological and HPLC data available for analysis, while molecular testing was performed in selected cases based on diagnostic indications.

Thalassemias constituted the predominant diagnostic category, accounting for the majority of cases evaluated. β-thalassemia trait was the most frequent diagnosis, followed by sickle cell trait, sickle cell disease, sickle–β-thalassemia, α-thalassemia, and β-thalassemia intermedia/major. Compound heterozygous hemoglobin variants were identified in a smaller proportion of cases (Table 1).

Table 1: Distribution of hemoglobinopathies diagnosed by laboratory evaluation.

Diagnosis	*Total (n)*	*%*	*Key HPLC findings*
β-thalassemia trait	124	44.0	↑ HbA2 (≥3.5%), normal/low HbF
β-thalassemia intermedia	18	6.4	↑ HbF, variable HbA2
β-thalassemia major	22	7.8	Markedly ↑ HbF, absent/low HbA
α-thalassemia	36	12.8	Normal HbA2 & HbF
Sickle cell trait	40	14.2	HbA > HbS
Sickle cell disease	24	8.5	HbS predominant, ↑ HbF
Sickle–β-thalassemia	12	4.3	HbS with ↑ HbA2/HbF
Other compound heterozygous states	6	2.0	Variant-dependent
Total	282	100	—

Note: Values shown as N and %. Comparison by Chi-square test; $p < 0.001$.

Hematological parameters showed significant variation across diagnostic categories. β-thalassemia trait cases demonstrated significantly higher RBC counts with reduced MCV and MCH compared to other groups ($p < 0.001$). In contrast, patients with β-thalassemia major and intermedia exhibited severe anemia with markedly elevated reticulocyte counts. α-thalassemia cases showed microcytosis with relatively preserved hemoglobin levels and normal HbA2 values, overlapping with iron deficiency and underscoring diagnostic challenges (Table 2).

Table 2: Comparison of hematological parameters across major diagnostic categories.

Parameter	*β-thal Trait*	*α-thalassemia*	*SCD*	*Sickle Trait*
Hemoglobin (g/dL)	10.8 ± 1.4	11.6 ± 1.2	8.2 ± 1.6	12.4 ± 1.1
RBC count ($\times 10^6/\mu L$)	5.6 ± 0.7	5.2 ± 0.6	3.1 ± 0.5	4.7 ± 0.6
MCV (fL)	64.2 ± 6.8	68.5 ± 5.9	84.6 ± 8.4	82.1 ± 6.7
MCH (pg)	19.6 ± 2.1	21.3 ± 2.0	27.5 ± 3.1	26.9 ± 2.4
RDW (%)	16.2 ± 2.4	15.8 ± 2.1	19.4 ± 3.2	14.8 ± 1.9

Note: Values expressed as mean ± SD. Comparison by one-way ANOVA; $p < 0.001$.

HPLC analysis revealed characteristic hemoglobin fraction patterns across different disorders. Elevated HbA2 levels were consistently observed in β-thalassemia trait, while increased HbF fractions were

noted in β-thalassemia major, intermedia, and sickle–β-thalassemia. Sickle cell trait and disease showed the expected HbS peaks, with quantitative differences aiding in differentiation. A statistically significant correlation was observed between HPLC findings and final diagnostic categorization ($p < 0.001$).

Molecular analysis was performed in diagnostically ambiguous cases, suspected carrier states, and compound heterozygous disorders. Molecular testing contributed to definitive diagnosis in 42 cases (14.9% of the total cohort), particularly in cases with borderline HbA2 levels and normal HPLC profiles. In 18 cases suspected of α-thalassemia based on microcytosis with normal HPLC, molecular testing confirmed deletional mutations, thereby altering the initial presumptive diagnosis. Additionally, in 10 cases of suspected sickle–β-thalassemia, molecular findings helped confirm compound heterozygosity and refine disease classification. Among β-thalassemia cases, the most frequently identified mutations were IVS-I-5 (G>C), Codon 41/42 (–CTTT), Codon 8/9 (+G), and the 619 bp deletion, while α-thalassemia was predominantly associated with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions. Molecular results showed strong concordance with hematological and HPLC findings ($p < 0.001$) and enabled definitive carrier identification in cases with borderline or normal HPLC profiles (Tables 3 and 4).

Table 3: Correlation of HPLC findings with final diagnosis.

HPLC Parameter	*β-thal Trait*	*α-thalassemia*	*SCD*	*Sickle–β-thal*
HbA2 elevated (>3.5%)	124 (100%)	0 (0%)	0 (0%)	12 (100%)
HbF elevated (>2%)	22 (17.7%)	0 (0%)	18 (75.0%)	10 (83.3%)
HbS detected	0 (0%)	0 (0%)	24 (100%)	12 (100%)
Normal HPLC	0 (0%)	36 (100%)	0 (0%)	0 (0%)

Note: Chi-square test; $p < 0.001$.

Table 4: Spectrum of molecular mutations identified in selected cases.

Mutation Type	*Number (n)*	*Percentage (%)*
β-thalassemia mutations		
IVS-I-5 (G>C)	22	36.7
Codon 41/42 (–CTTT)	14	23.3
Codon 8/9 (+G)	10	16.7
619 bp deletion	8	13.3
Others	6	10.0
α-thalassemia deletions		
$-\alpha^{3.7}$	18	60.0
$-\alpha^{4.2}$	12	40.0

Note: Values shown as N and % of tested cases.

Overall, integration of hematological indices, HPLC patterns, and molecular testing significantly enhanced diagnostic accuracy, particularly in carrier detection and complex hemoglobinopathy states.

Discussion

This study highlights the predominance of thalassemias among hemoglobinopathies encountered in a tertiary care setting in India, reaffirming their significant public health impact in this region [12, 13]. The predominance of β-thalassemia trait in the study cohort is consistent with earlier Indian studies and reflects the high carrier frequency reported across diverse ethnic and geographic populations in the country [2, 3]. The inclusion of all consecutive patients evaluated over a one-year period, without preselection or duplication, ensured a representative assessment of the diagnostic spectrum encountered in routine laboratory practice.

Distinct and statistically significant differences in hematological parameters across diagnostic categories underscore the continued relevance of red cell indices as the first-line screening tool for hemoglobinopathies. Elevated RBC counts with reduced MCV and MCH in β-thalassemia trait, and severe anaemia with reticulocytosis in β-thalassemia major and intermedia, were consistent with established hematological profiles [14]. However, the overlap of microcytosis between α-thalassemia carriers, β-thalassemia trait, and iron deficiency anaemia reiterates the limitations of hematological parameters alone and the risk of underdiagnosis, particularly for α-thalassemia [15].

HPLC proved to be a robust and reliable modality for hemoglobin variant analysis, enabling accurate quantification of HbA2 and HbF and facilitating differentiation of common hemoglobinopathies [7, 8]. Elevated HbA2 levels were a consistent finding in β-thalassemia trait, while characteristic HbS fractions aided in the identification of sickle cell disorders and sickle–β-thalassemia. Nonetheless, normal or borderline HPLC profiles observed in α-thalassemia carriers and some compound heterozygous states emphasize the

inherent diagnostic limitations of HPLC when used in isolation [9].

Importantly, molecular testing demonstrated significant incremental diagnostic value in this study. It enabled definitive classification in cases with equivocal hematological and HPLC findings, particularly in α-thalassemia carriers and compound heterozygous states. In a subset of cases, molecular analysis led to reclassification of diagnosis, underscoring its role not only as a confirmatory tool but also as a decisive modality in complex scenarios.

The integration of molecular testing significantly enhanced diagnostic precision, particularly in cases with equivocal hematological and HPLC findings. The predominance of IVS-I-5 (G>C), Codon 41/42 (–CTTT), Codon 8/9 (+G), and the 619 bp deletion among β-thalassemia cases, along with $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions in α-thalassemia, mirrors the known mutation spectrum of the Indian population[6]. The strong concordance observed between molecular results and conventional laboratory parameters reinforces the value of targeted mutation analysis as a confirmatory and problem-solving tool in routine practice.

From a public health perspective, the high proportion of carrier states identified in this study underscores the critical importance of systematic carrier screening and genetic counseling in India. Early and accurate identification of carriers enables informed reproductive choices and forms the cornerstone of effective prevention strategies aimed at reducing the burden of transfusion-dependent thalassemia. The study findings support a tiered diagnostic approach, wherein hematological screening and HPLC serve as initial tools, followed by molecular confirmation in selected cases.

Limitations: This study has certain limitations. The ambispective design and single-center setting may limit the generalizability of findings to other populations. Molecular testing was performed selectively rather than uniformly across all cases, which may introduce selection bias and lead to underrepresentation of the true mutation spectrum. In addition, iron studies were not uniformly available for all patients, which could confound the interpretation of microcytic anaemia, particularly in differentiating iron deficiency from thalassemia traits. Furthermore, the sample size for certain subgroups, such as compound heterozygous states, was relatively small, thereby limiting detailed subgroup analysis.

Conclusion

This study reinforces the central role of integrated laboratory evaluation in the diagnosis of hemoglobinopathies in the Indian population and highlights thalassemias as the most prevalent disorders encountered in tertiary care practice. While hematological indices and HPLC provide reliable initial classification, molecular testing is essential for definitive diagnosis in selected cases, particularly for carrier detection and complex hemoglobinopathy states. These findings underscore the importance of comprehensive screening strategies to support effective prevention, genetic counselling, and reduction of disease burden in India.

Conflicts of interest

Authors declare no conflicts of interest.

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