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Diagnostic patterns of haemoglobinopathies: A prospective observational study using high-performance liquid chromatography in a remote tertiary care center of Telangana.

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Abstract

Background
Haemoglobinopathies, including sickle cell disorders and thalassemias, are significant genetic conditions that alter hemoglobin structure and function. High-performance liquid chromatography is a sensitive and reliable tool for identifying hemoglobin variants. This study aimed to analyze the diagnostic patterns of haemoglobinopathies using HPLC in a tertiary care center serving the tribal population of Telangana.

Methods
A prospective observational study was conducted over 18 months (June 2023 to December 2024) involving 182 patients attending a tertiary care hospital. Hemoglobin variants—Hb A0, Hb A2, Hb F, and Sickle Cell Window—were analyzed using HPLC. Red cell distribution width (RDW-CV) was assessed to evaluate erythrocyte size variability. Diagnostic classification was based on HPLC parameters, including specific thresholds for sickle cell disease, trait, and beta-thalassemia.

Results
Among the 182 cases, 6 (3.3%) were diagnosed with sickle cell disease (S-window >40%), 62 (34.1%) with sickle cell trait (S-window <40%), and 9 (4.9%) with probable sickle cell beta-thalassemia trait (S-window <40% and Hb A2 between 3.5–4.5%). The remaining 105 cases (57.7%) were unclassified on HPLC alone but showed features suggestive of iron deficiency anemia, anemia of chronic disease, or borderline hemoglobinopathy patterns. Mean values observed were: Hb A0 = 71.77%, Hb A2 = 2.81%, Hb F = 2.11%, and Sickle Cell Window = 36.58%. RDW-CV was highest in sickle cell disease cases (mean 17.27%) compared to the trait (15.35%).

Conclusion
This study demonstrates a high burden of sickle cell disorders among the tribal population in Telangana. HPLC proves to be a valuable diagnostic modality, but further molecular testing is essential for accurate classification, particularly in compound and borderline cases. Early identification, genetic counseling, and targeted screening are vital for managing haemoglobinopathies in high-risk regions.

Recommendations
Implement universal newborn screening, strengthen genetic counseling services, enhance molecular diagnostics, and promote community awareness for effective haemoglobinopathy management.

Keywords: *Haemoglobinopathy, Sickle cell disorder, High-Performance Liquid Chromatography, Sickle cell beta-thalassemia, Hemoglobin variants, Tribal population, Genetic screening*
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Introduction

Haemoglobinopathies are a diverse group of inherited disorders resulting from structural abnormalities in the hemoglobin molecule or imbalanced synthesis of its globin chains. Among these, sickle cell disease and thalassemias are the most clinically significant, contributing to considerable global morbidity and mortality [1]. The burden of these disorders is particularly high in developing countries, including India, where genetic, geographical, and socio-environmental factors contribute to a higher prevalence in specific populations—especially among tribal communities [1,2].

In India, sickle cell disorders are disproportionately prevalent in tribal populations, with reported rates ranging from 1% to over 40% depending on the region [3]. Telangana, a state with a sizeable tribal demographic, is recognized as a high-risk zone for haemoglobinopathies. However, due to limited access to advanced diagnostic infrastructure in remote healthcare settings, many affected individuals remain undiagnosed or misclassified [4].

High-performance liquid chromatography (HPLC) has emerged as a preferred diagnostic modality for the identification and quantification of hemoglobin variants [2,5]. It provides several advantages over traditional techniques such as electrophoresis, including improved sensitivity, automation, reproducibility, and the ability to resolve complex and coexisting hemoglobin disorders [3,6]. HPLC is particularly useful for diagnosing common conditions like sickle cell disease and beta-thalassemia, while also facilitating the detection of less common variants such as Hb D, Hb E, and compound heterozygous states [5,6].

The Thalassaemia International Federation has emphasized the role of standardized laboratory protocols, including HPLC, as essential components in the prevention and management of hemoglobinopathies through early diagnosis, carrier detection, and genetic counseling [7].

This study was undertaken to examine the spectrum of haemoglobinopathies diagnosed via HPLC in a tertiary healthcare facility predominantly catering to the tribal communities of Telangana. By evaluating the distribution of hemoglobin variants and associated red cell indices, particularly red cell distribution width (RDW), this research seeks to improve early identification, classification, and management strategies for haemoglobinopathies in resource-limited and high-risk regions.

Methodology

Study design and setting

A prospective observational study was conducted over a period of 18 months, from June 2023 to December 2024, at the Government Medical College and General Hospital, Bhadradri Kothagudem, Telangana. This tertiary care center predominantly serves the tribal population residing in the remote and underserved regions of Telangana, making it a crucial hub for the diagnosis and management of genetic and hematological disorders.

Study population

The study included 182 patients who were referred to the hematology department for evaluation of anemia, suspected hemoglobinopathies, or other hematological abnormalities. All patients presenting during the study period and undergoing hemoglobin variant analysis were considered for inclusion, regardless of age or sex.

Sample Size Determination

The study employed a convenience sampling method, enrolling all patients referred for hemoglobinopathy evaluation over 18 months. A total of 182 cases were included, reflecting the real-time diagnostic burden in this high-prevalence tribal region and ensuring adequate representation across different hemoglobinopathy patterns.

Inclusion criteria

- Patients of all age groups and genders
- Individuals with clinical suspicion of hemoglobinopathy (e.g., anemia, jaundice, splenomegaly, family history)
- Patients who provided informed consent for testing and participation

Exclusion criteria

- Patients with a recent blood transfusion (within the last 3 months)
- Incomplete or poor-quality HPLC chromatograms
- Patients with known coexisting hematologic malignancies or chronic inflammatory conditions affecting hemoglobin levels

Data collection and diagnostic procedures

Peripheral venous blood samples (2 mL) were collected under aseptic precautions in EDTA tubes. All samples

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were subjected to high-performance liquid chromatography (HPLC) using the Bio-Rad Variant™ II Hemoglobin Testing System, a standardized and automated platform for hemoglobin variant analysis.
The following hemoglobin fractions were quantitatively assessed:
Hemoglobin A0 (Hb A0)
Hemoglobin A2 (Hb A2)
Fetal Hemoglobin (Hb F)
Sickle Cell Window (Hb S or related variants)
Additionally, Red Cell Distribution Width – Coefficient of Variation (RDW-CV) values were obtained from automated hematology analyzers to assess anisocytosis, which is particularly relevant in hemolytic conditions like sickle cell disease.

Diagnostic classification

Based on HPLC results, patients were classified into the following categories:
Sickle Cell Disease (SCD): Sickle Cell Window >40%, with or without elevated Hb F, and reduced or absent Hb A0
Sickle Cell Trait (SCT): Sickle Cell Window <40%, with coexisting Hb A0
Sickle Cell Beta-Thalassemia Trait (likely): Sickle Cell Window <40% combined with Hb A2 levels between 3.5% and 4.5%, and reduced Hb A0
Other Haemoglobinopathies: Variations in Hb A2 (>3.5%) and/or Hb F (>2%) suggestive of beta-thalassemia trait or persistent fetal hemoglobin
Unclassified Cases: Profiles not meeting the above thresholds, possibly due to iron deficiency, anemia of chronic disease, or borderline/bicomponent traits
Where indicated, clinical correlation and further molecular diagnostics were recommended to confirm complex or compound hemoglobinopathy states.

Bias control

To minimize selection bias, all eligible patients presenting during the study period were consecutively enrolled. Standardized protocols were followed for sample collection and HPLC analysis to reduce measurement bias. Interpretation was performed independently by two pathologists to avoid observer bias and ensure diagnostic consistency.

Ethical considerations

Ethical approval for the study was obtained from the Institutional Ethics Committee of Government Medical College, Bhadradri Kothagudem. Written informed consent was secured from all participants or legal guardians. Strict confidentiality of patient data was maintained throughout the study.

Results

Participants

Out of 192 patients referred to the hematology department during the study period, 186 were initially screened. Four were excluded due to recent blood transfusion or incomplete HPLC data. The final analysis included 182 participants who fulfilled all eligibility criteria and had interpretable HPLC chromatograms. All 182 completed testing and were included in the final analysis.

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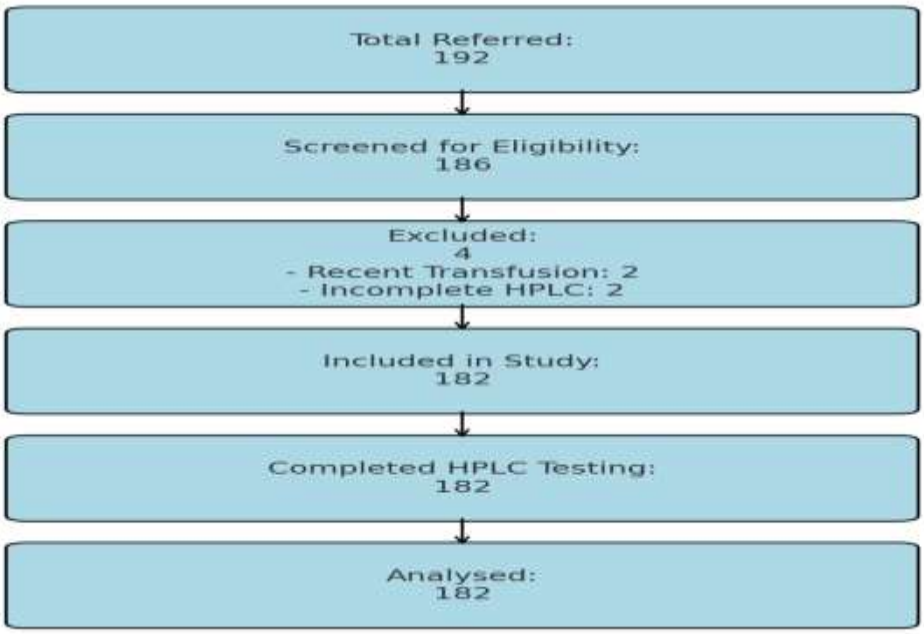

Figure 1: Participant flow diagram

Patient demographics

A total of 182 patients were included in the study, comprising individuals from all age groups, with a balanced gender distribution. All patients underwent hemoglobin variant analysis using high-performance liquid chromatography (HPLC).

Table 1. Demographic and clinical characteristics of study participants (N = 182)

Characteristic	Category	Frequency (n)	Percentage (%)
Age Group (years)	<10	18	9.9
	11–20	43	23.6
	21–30	52	28.6
	31–40	29	15.9
	>40	40	22.0
Gender	Male	94	51.6
	Female	88	48.4
Tribal Affiliation	Yes	156	85.7
	No	26	14.3
Clinical Features	Anemia	141	77.5
	Jaundice	29	15.9
	Splenomegaly	22	12.1
Family History	Positive for hemoglobinopathy	31	17.0

Diagnostic classification based on HPLC

The initial HPLC-based classification identified 6 cases (3.3%) as sickle cell disease (SCD) and 62 cases (34.1%) as sickle cell trait (SCT). A substantial number of cases (114, 62.6%) were originally categorized as unclassified due to non-definitive patterns(Table 2).

Upon applying stricter HPLC-based criteria and clinical interpretation:

Sickle Cell Disease (SCD) was confirmed in patients with a sickle cell window >40%, along with low or absent Hb A0 levels and elevated Hb F.

Sickle Cell Trait (SCT) cases demonstrated sickle cell window values <40% with a concurrent presence of Hb A0.

Sickle Cell Beta-Thalassemia Trait was inferred in approximately **5% (n=9)** of unclassified cases based on

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the presence of sickle cell window values around 35–40%, mildly elevated Hb A2 (>3.5%), and reduced Hb A0.
The remaining unclassified cases (approximately 105 patients) showed HPLC profiles and red cell indices suggestive of:

- **Iron deficiency anemia**
- **Anemia of chronic disease**
- **Borderline thalassemic traits or non-specific patterns** not meeting definitive diagnostic thresholds.

This reclassification enhances diagnostic clarity and emphasizes the need for additional confirmatory investigations, such as iron studies and molecular diagnostics.

Table 2: Haemoglobinopathy classification

Diagnosis	Number of Cases	Percentage
Sickle Cell Disease	6	3.3%
Sickle Cell Trait	62	34.1%
Sickle Cell Beta-Thalassemia Trait (likely)	9	4.9%
Iron Deficiency/Chronic Disease/Other Unclassified	105	57.7%
Total	**182**	**100%**

The distribution of hemoglobin fractions across the study population is summarized in Table 3. The mean Hb A0 level was 71.77%, with a broad range of values (2.2% – 88.5%), indicating normal and abnormal hemoglobin production. Hb A2 levels showed a mean of 2.81%, with some cases exceeding 3.5%, suggesting possible beta-thalassemia traits. The Hb F levels had a mean of 2.11%, with some cases exhibiting significantly higher values (up to 28.3%), indicative of potential fetal hemoglobin persistence.

Table 3: Summary of hemoglobin levels

Parameter	Count	Mean	Std Dev	Min	25%	50%	75%	Max
RDW-CV (%)	171	15.73	2.47	12.4	14.05	15.1	16.8	25.2
Hb A0 Level (%)	177	71.77	19.58	2.2	58.3	83.8	85.4	88.5
Hb A2 Level (%)	177	2.81	1.99	0.9	2.3	2.7	3.0	27.4
Hb F Level (%)	140	2.11	4.50	0.1	0.78	0.90	1.30	28.3
Sickle Cell Window (%)	65	36.58	12.14	23.9	28.8	34.3	38.6	77.8

The Sickle Cell Window values, critical for diagnosing sickle cell disorders, had a mean of 36.58%, with cases ranging from 23.9% to 77.8%. These findings confirm the presence of sickle cell disease and trait in a substantial proportion of the population.

The RDW-CV values ranged from 12.4% to 25.2%, with a mean of 15.73%, indicating variations in red blood cell size. Higher RDW values were observed in cases of sickle cell disease (mean 17.27%), compared to sickle cell trait (mean 15.35%) and unclassified cases (mean 15.86%), as shown in Table 4.

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Table 4: RDW-CV analysis by diagnosis

Diagnosis	Count	Mean	Std Dev	Min	25%	50%	75%	Max
Sickle Cell Disease	6	17.27	2.46	14.6	15.73	16.85	18.05	21.5
Sickle Cell Trait	60	15.35	1.96	12.5	14.08	14.70	16.13	20.7
Unclassified Cases	105	15.86	2.69	12.4	13.80	15.40	16.70	25.2

The significantly increased RDW in sickle cell disease cases suggests a higher degree of anisocytosis, consistent with hemolytic anemia.

Discussion

This prospective study highlights the diagnostic spectrum of haemoglobinopathies using high-performance liquid chromatography (HPLC) among a predominantly tribal population. The results demonstrate a considerable burden of sickle cell disorders, with 3.3% diagnosed with sickle cell disease and 34.1% with sickle cell trait—findings that are consistent with regional data from southern and central India, where the prevalence of sickle cell trait ranges between 10% and 35% [8,10,14].

HPLC proved to be a highly effective diagnostic modality for quantifying hemoglobin variants and distinguishing between carrier and disease states. The mean Hb A0 level (71.77%) and Sickle Cell Window value (mean 36.58%) enabled accurate identification of sickle cell patterns, reinforcing the tool's utility as established in previous Indian cohorts [8,10,11]. The elevated RDW-CV values in sickle cell disease cases (mean 17.27%) reflect significant anisocytosis due to ongoing hemolysis, aligning with literature that correlates RDW increases with disease severity and red cell morphological variability [8,11].

A key challenge identified was the substantial proportion of unclassified cases (62.6%). This may be attributed to borderline hemoglobin variant levels or coexisting nutritional deficiencies, such as iron deficiency or chronic inflammation, that can alter red cell indices and mask hemoglobinopathy patterns [11,13]. These limitations have been echoed in earlier studies, underscoring the need for adjunct molecular investigations such as gap-PCR, DNA sequencing, or globin gene analysis to detect compound heterozygous states and rare variants like Hb D-Punjab or Hb Q-India [12].

The mean Hb A2 level (2.81%) was within the normal range for most participants; however, a subset of cases exhibited levels above 3.5%, suggesting a possible beta-thalassemia trait. Among these, cases with Hb A2 values between 3.5% and 4.5% along with sickle cell window values below 40% were suggestive of Sickle Cell Beta-Thalassemia Trait - a compound heterozygous state that is increasingly recognized in Indian tribal populations. These results underscore the advanced utility of HPLC in detecting thalassemia carrier states with greater diagnostic precision. Nevertheless, elevated Hb A2 alone is not conclusive, particularly in the context of coexisting iron deficiency, and must be interpreted in conjunction with red cell indices, clinical history, and family studies [9,11]. Comparable studies from rural and tribal areas of West Bengal, Maharashtra, and Pakistan report similar diagnostic dilemmas and prevalence patterns, further reinforcing the need for localized data to inform region-specific screening strategies [10,13,14]. These results emphasize the critical importance of comprehensive and integrated public health approaches, including genetic counseling, antenatal screening, and community awareness campaigns, to effectively manage hemoglobinopathies in underserved tribal regions [9,13].

Generalizability

The findings of this study are primarily applicable to tribal and underserved populations in Telangana and similar regions with a high prevalence of haemoglobinopathies. While the use of standardized HPLC protocols enhances reproducibility, the results may not be fully generalizable to urban or non-tribal settings without further multicentric studies and broader demographic representation.

Conclusion

This study demonstrates a significant prevalence of sickle cell disorders, with sickle cell trait being notably common. High-performance liquid chromatography (HPLC) proved to be an effective tool for detecting and quantifying hemoglobin variants, facilitating the diagnosis of haemoglobinopathies. However, the large proportion of unclassified cases highlights the limitations of HPLC alone and underscores the need for supplementary genetic testing. The elevated RDW-CV values in sickle cell disease cases further support its role as a marker of red cell variation. Early diagnosis, community screening, and genetic counseling are essential for effective disease control and public health planning in vulnerable populations.

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Limitations

This study was limited by the lack of molecular confirmatory testing, which restricted definitive diagnosis in borderline and compound cases. Additionally, the sample size was relatively small, and the study was confined to a single tertiary center, limiting the generalizability of findings to the broader tribal population of Telangana.

Recommendations

To effectively address the burden of haemoglobinopathies in tribal regions, it is recommended to implement universal newborn screening programs for early detection. Strengthening genetic counseling services is essential to inform affected families about inheritance patterns and reproductive options. Expanding access to molecular diagnostic facilities will enable accurate classification, especially in compound or borderline cases. Regular community-based awareness campaigns should be conducted to educate the population on the importance of early diagnosis and preventive measures. Additionally, integrating haemoglobinopathy screening into routine antenatal care can help in identifying at-risk couples and reducing disease transmission through informed family planning and early interventions.

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List of abbreviations

Hb – Hemoglobin
Hb A0 – Hemoglobin A0
Hb A2 – Hemoglobin A2
Hb F – Fetal Hemoglobin
HPLC – High-Performance Liquid Chromatography
RDW-CV – Red Cell Distribution Width – Coefficient of Variation
SCD – Sickle Cell Disease
SCT – Sickle Cell Trait
EDTA – Ethylenediaminetetraacetic Acid
DNA – Deoxyribonucleic Acid
PCR – Polymerase Chain Reaction

Source of funding

The study had no funding.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

SM-Concept and design of the study, results interpretation, review of literature, and preparing the first draft of the manuscript. Statistical analysis and interpretation, revision of manuscript.

CSB-Concept and design of the study, results interpretation, review of literature, and preparing the first draft of the manuscript, revision of the manuscript.

KR-Review of literature and preparing the first draft of the manuscript. Statistical analysis and interpretation.

Data availability

Data available on request

Author biography

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Dr. Raja Kumar is currently serving as the Principal and Professor of Pathology at Government Medical College, Bhadradri Kothagudem, Telangana. Before this, he held various academic positions, including Assistant Professor at KMC Warangal and Associate Professor at Government Medical College, Suryapet, contributing significantly to the growth of both institutions through his commitment to teaching and leadership. He has published extensively in reputed international journals and continues to contribute to medical research, with a special focus on pathology and medical education. Dr. Raja Kumar is known for his dedication to student development, institutional advancement, and the promotion of evidence-based pathology practices in clinical settings. **ORCID ID:** https://orcid.org/0009-0006-8550-0224

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