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Original Research Article

A SPECTRUM OF HEMOGLOBINOPATHIES USING HPLC: OBSERVATIONAL STUDY AMONG NORTH INDIANS

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ABSTRACT

Background: High-Performance Liquid Chromatography (HPLC) is a widely used method for diagnosing hemoglobinopathies. This study aims to evaluate hemoglobin variants among 100 patients using HPLC over a two-year period. The study aims to evaluate the prevalence and spectrum of hemoglobinopathies among North Indian individuals using High-Performance Liquid Chromatography (HPLC) as the diagnostic modality. It also compares key hematological parameters with findings from other published studies.

Materials and Methods: We retrospectively analyzed 100 patient samples processed via HPLC between January 2023 and December 2024. Parameters assessed included HbA1, HbA2, HbF, HbS, and other variant peaks, along with demographic and hematological indices.

Results: Of the 100 patients, several hemoglobinopathies were identified, including β-thalassemia trait, HbE trait, sickle cell trait etc. HbA2 values >3.5% were strongly associated with β-thalassemia. Approximately 5% showed elevated HbF levels, while 2% had identifiable HbS. A correlation was noted between variant hemoglobins and red cell indices such as MCV and MCH.

Conclusion: HPLC remains a vital diagnostic tool for detecting and characterizing hemoglobinopathies. Our data support its routine use in clinical screening and diagnostic settings, especially in regions with a high prevalence of hemoglobin variants.

Keywords: Hemoglobinopathies, β-Thalassemia, High-Performance Liquid Chromatography, HbA2, HbF, India.

INTRODUCTION

Hemoglobinopathies are inherited disorders affecting the structure or production of the hemoglobin molecule, primarily arising due to mutations in the globin genes. These disorders include both quantitative defects (e.g., thalassemias) and qualitative abnormalities (e.g., sickle cell disease, HbE, HbD) that alter the hemoglobin's function or stability. Globally, hemoglobinopathies account for a significant portion of morbidity due to genetic diseases, and early detection is essential for effective management, genetic counseling, and disease prevention programs.[1]

Thalassemia syndromes, particularly beta-thalassemia, are caused by mutations in the HBB gene leading to reduced or absent beta-globin chain production. The disease spectrum ranges from asymptomatic carriers to transfusion-dependent thalassemia major. India contributes approximately 10% of the global thalassemia burden, with a carrier rate estimated between 3% and 4% in the general population, though this varies regionally.[2,3]

Sickle cell disease (SCD), caused by a point mutation in the β-globin gene (Glu6Val), leads to the production of hemoglobin S (HbS). Under hypoxic conditions, HbS polymerizes, causing erythrocyte deformation, hemolysis, and vaso-occlusive crises. Though traditionally associated with African ancestry, sickle cell trait and disease are now recognized in several Indian and Mediterranean populations due to gene flow and migration patterns.[4]

High-Performance Liquid Chromatography (HPLC) has emerged as a preferred method for screening hemoglobinopathies because of its speed,

automation, high sensitivity, and ability to quantify hemoglobin fractions such as HbA2 and HbF. Elevated HbA2 (>3.5%) is a diagnostic hallmark of β-thalassemia trait, while significant HbS or HbE fractions signal the presence of structural variants.[5,6] Several recent studies across India have established the efficacy of HPLC in detecting a wide spectrum of hemoglobinopathies. For instance, Chakma et al. (2022) analyzed over 100 samples in North India and demonstrated the accurate identification of abnormal variants using HPLC.[7] Similarly, Bhuvana et al. (2022) emphasized the importance of integrating HPLC screening in routine diagnostic workflows for early detection and genetic counselling.[8]

This study seeks to fill that gap by analyzing HPLC-derived hemoglobin profiles from a North India cohort to evaluate the prevalence and spectrum of hemoglobinopathies, focusing on beta-thalassemia and sickle cell traits. These findings can help guide public health policy on carrier screening and thalassemia prevention strategies in multi-ethnic populations.

MATERIALS AND METHODS

Study Design: A retrospective, cross-sectional analysis of HPLC data collected from 100 patients between January 2023 and December 2024.

Sample Collection: Peripheral blood samples were collected in EDTA vacutainers and analyzed within 24 hours of collection.

HPLC Analysis: Samples were processed using a Bio-Rad Variant II HPLC system. Hemoglobin fractions measured included HbA1a, HbA1b, HbF, HbA2, HbS, and others.

Inclusion Criteria:

- Patients of all age groups and both sexes.
- Samples with complete demographic and hematological parameters.

Exclusion Criteria:

- Hemolyzed or clotted samples.
- Incomplete records.

Data Analysis: Descriptive statistics were used to analyze the distribution of hemoglobin variants. Hematological indices like MCV, MCH, RBC count, and Hb levels were compared across variant and normal groups.

RESULTS

A total of 100 patient samples were analyzed using HPLC between January 2023 and December 2024. The following findings were observed:

Demographic Distribution

- **Age:** Patients ranged from 6 months to 72 years (mean age: 32.4 ± 15.6 years).
- **Gender:** 52% were males (n=52) and 48% were females (n=48).
- **OPD Distribution:** Patients were referred from routine checkups, antenatal clinics, and suspected anemia clinics.

HPLC Findings

Statistical Summary of Hemoglobinopathy Interpretations (n = 100)

Hemoglobinopathy	Count	Percentage (%)
HbE Trait	03	3.0%
HbD Punjab	01	1.0%
Beta Thalassemia minor	09	9.0%
Beta Thalassemia major	02	2.0%
Normal	82	82.0%
Sickle Cell Trait	02	2.0%
HbD Punjab + Beta Thalassemia Trait	01	1.0%

An unknown peak at retention time of 3.78 with 39% area was identified in a patient. The same patient showed another peak of HbA2 having 4.5% area . This patient was classified under the category of HbD Punjab+Beta Thalassemia trait.

- Most Common Abnormalities:
- Beta Thalassemia minor (9%)
- HbE Trait (3%)
- Sickle Cell Trait (2%)

Table 1: Summary of Hematological Parameters by HPLC Variant Diagnosis

Interpretation	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (×10⁶/µL)	HbF(%)	HbA2 (%)	HbS (%)
Beta Thalassemia Trait	10.2	63.21	21.76	29.78	5.84	0.9	4.34	0
Beta Thalassemia Major	5.10	59.20	19.10	27.22	2.95	67.78	3.1	0
HbD Punjab + Beta Thal Trait	10.55	64.30	21.98	30.19	6.05	0.9	4.50	0
HbE Trait	11.12	77.68	23.33	32.21	5.97	1.45	27	0
Sickle Cell Trait	10.13	75.73	26.46	31.77	5.09	1.77	3.5	32
Normal	12.1	80.64	27.1	32.2	4.19	0.98	2.3	0

Table 2: Comparison of between various studies for Beta thallasemia minor

Parameter	Present Study	Chennai (2011)Wiley(13)	Ahmedabad (2021)(14)	Eastern India(15)	Uttarakhand (2023)(16)
Hb (g/dL)	10.2	10.4	10.5	10.4	10.1
MCV (fL)	63.2	65.5	64.2	65.5	64.3
MCH (pg)	21.8	19.9	21.0	19.9	20.4
MCHC (g/dL)	29.8	30.5	31.0	30.5	30.2
RBC (×10⁶/µL)	5.84	5.2	5.4	5.2	5.6
HbF (%)	1.32	1.4	1.2	1.4	1.3
HbA_2 (%)	4.34	5.4	5.2	5.3	≥4.0 (diagnostic cut-off)

Table 3: Comparison of between various studies for Beta thallasemia major

Parameter	Present Study	Chennai (2011)Wiley	Ahmedabad (2021)	Eastern India	General Indian Range
Hb (g/dL)	5.10	4.8	5.5	5.3	<7
MCV (fL)	59.2	67.9	65.8	66.4	50–70
MCH (pg)	19.1	21.1	20.3	19.8	12–22
MCHC (g/dL)	27.2	31.0	30.4	29.6	28–32
RBC (×10⁶/µL)	2.95	2.4	2.8	2.6	Low (<3)
HbF (%)	67.78	88.0	82.3	85.1	70–95
HbA_2 (%)	6.70	~7.2 (with no HbA)	~6.5	6.8	>5

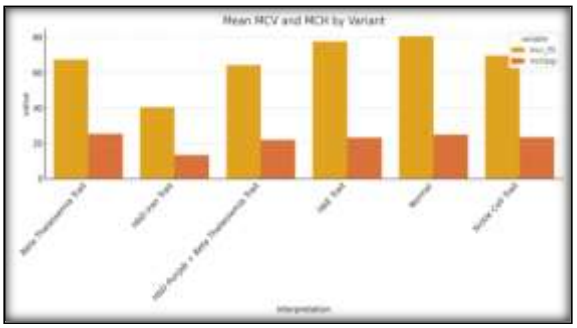

Figure 2: Bar Chart – Mean MCV and MCH by Variant

DISCUSSION

The comparison of β-thalassemia minor and major cases across the present study and four Indian HPLC-based studies reveals strong concordance in hematological and chromatographic parameters. In β-thalassemia minor, hemoglobin levels were consistently mild-to-moderately reduced (10.2 g/dL in the present study vs. ~10.4–10.5 g/dL across Indian studies), with uniform microcytosis and hypochromia reflected by low MCV (63.2–65.5 fL) and MCH values (~20–22 pg). HbA_2 levels, a key diagnostic marker, were elevated in all minor cases (4.34% in the current dataset, compared to 5.2–5.4% in the Chennai and Ahmedabad studies), confirming the trait status. HbF showed a mild increase in minors (1.3–1.4%), consistent with published Indian data.

In contrast, β-thalassemia major cases demonstrated classic features of severe anemia (Hb ~5 g/dL), marked microcytosis (MCV ~59–68 fL), low RBC counts (~2.4–2.95 ×10⁶/µL), and a dramatic rise in HbF levels (67.8% in the present study vs. 85–88% in Chennai and Eastern Indian cohorts). HbA_2 was also elevated (>6%) in major cases, though its diagnostic value is secondary to the markedly high HbF. The close alignment between the present data and regional studies underscores the reliability of HPLC in diagnosing and characterizing thalassemia syndromes across diverse Indian populations.

HbD Punjab: Hemoglobin D Punjab is a β-globin variant caused by a mutation at codon 121 (Glu→Gln), commonly seen in North India and Pakistan. It is usually asymptomatic in the heterozygous state, with normal or mildly altered red cell indices. On HPLC, it shows a distinct peak in the D window. While clinically silent alone, it can cause symptoms if co-inherited with other variants like HbS or β-thalassemia.

HbD Punjab + Beta Thalassemia Trait: This compound heterozygous condition was identified in one patient. The HPLC pattern showed prominent HbD and HbA2 peaks. The patient had microcytic anemia with moderately elevated RBC count. While HbD Punjab has sickling potential and Beta Thalassemia Trait causes quantitative beta chain defects, the co-inheritance can lead to variable phenotypic expression. Careful distinction from sickling disorders is essential for genetic counseling.

β-Thalassemia minor: The most frequently detected abnormality was β-thalassemia minor (9%), which is consistent with regional and national data from high-prevalence zones in India. Elevated HbA2 (>3.5%) remains the hallmark of diagnosis. These cases often presented with microcytic hypochromic anemia, reflected in decreased MCV and MCH, while maintaining near-normal Hb levels in some.

Sickle Cell Trait (2%)

Patients with sickle cell trait displayed characteristic HbS peaks on HPLC. Most had heterozygous expression, with HbS levels between 25–45%. Despite the absence of sickle cell disease symptoms, mild anemia was seen

The ethnic and geographic background of these patients, particularly from North Indian populations, further supports the clustering of sickle cell trait in these regions.

HbE Trait (3%)

HbE disorders, although less common in north India, were detected in 3% of the patients. HPLC efficiently identified HbE co-elution with HbA2, supporting

previous evidence of its diagnostic capability. Normal haemoglobin levels and mild microcytosis were common in these patients, emphasizing the need to differentiate HbE trait from iron deficiency and thalassemia.

Elevated HbF Patterns

Elevated fetal hemoglobin (HbF >2%) was observed in 5 patients. This increase could be attributed to hereditary persistence of fetal hemoglobin (HPFH), β-thalassemia intermedia, or stress erythropoiesis. HbF was increased in Beta thallasemia major, Persistence of fetal haemoglobin, Sickle cell disease and Beta thallasemia minor

6 month baby and HbF

By around 6 months of age, HbF typically falls to 10–40%, though values can vary depending on genetic background and health status. Persistently elevated HbF beyond this age may suggest underlying hemoglobinopathies such as β-thalassemia major, hereditary persistence of fetal hemoglobin (HPFH), or other conditions affecting hemoglobin switching

Comparison of various studies

In the present study, the HbF level in patients with Beta Thalassemia Major was 67.78%, which, although significantly elevated, was found to be lower compared to other reported values. Ruchi et al. (2017) and Chandrashekar et al. (2015) reported HbF levels of 85% and 86.4%, respectively. Patel et al. (2021) observed even higher levels (92.3%), while Kumar et al. (2019) documented a similar elevation at 88.6%. These differences might be attributed to variable transfusion histories, patient age, or co-existing hemoglobinopathies. Hemoglobin levels remained consistently low across all studies, supporting the profound anemia typical of this disorder. MCV and MCH values also aligned with microcytic, hypochromic indices, although slight inter-study differences existed.

Clinical Relevance: Routine use of HPLC allows not only diagnosis but also carrier detection, genetic counseling, and targeted public health interventions. Unlike electrophoresis, HPLC offers quantification of minor fractions like HbA2 and HbF, which is essential for confirming borderline or silent carriers.

Study Strengths and Limitations

Strengths:

- Use of a robust, validated HPLC method.
- Analysis of both hemoglobin variants and hematological indices.
- Inclusion of real-world clinical samples.

Limitations:

- Lack of molecular diagnostic confirmation for atypical or unknown peaks.
- No clinical follow-up data to correlate with HPLC findings.
- Potential regional bias due to single-center data collection.

Comparison with national data (e.g., Mondal et al., Bhuvana et al.) shows consistent trends in HPLC-based HbA2 and HbF quantification. Mean HbA2 values in β-thalassemia trait patients were aligned with international thresholds (>3.5%).[11,12]

CONCLUSION

This study highlights the utility of HPLC in the detection and differentiation of hemoglobinopathies in North India. The significant prevalence of β-thalassemia trait and sickle cell disorders underscores the need for population-level screening and genetic counseling. Implementation of routine HPLC screening, particularly in antenatal programs, is critical for reducing the incidence of thalassemia major and SCD in future generations.

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