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Original Research Article

Spectrum of Hemoglobinopathies Diagnosed by High-Performance Liquid Chromatography (HPLC) at a Tertiary Care Centre in Andaman and Nicobar Islands: A Retrospective Study

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Conflict of interest: Nil

Abstract:

Background: Hemoglobinopathies are inherited disorders affecting hemoglobin synthesis and structure and represent an important public health concern in India. High-Performance Liquid Chromatography (HPLC) is widely used for rapid screening and identification of hemoglobin variants. This study was undertaken to determine the spectrum of hemoglobinopathies diagnosed by HPLC at a tertiary care centre in the Andaman and Nicobar Islands.

Methods: A retrospective observational study was performed in the Department of Pathology, ANIIMS & GB Pant Hospital, Port Blair for a Period of one year. HPLC records from 1 April 2025 to 31 March 2026 were reviewed. Demographic details, hemoglobin concentration, HPLC fractions and final HPLC diagnosis were analysed. Hemoglobin fraction analysis was performed on a Lifotronic HPLC analyzer. Descriptive statistics were used.

Results: A total of 156 cases were analysed. Age was recorded in 145 cases, with a mean age of 23.64 ± 15.42 years. Sex was available for 148 cases. Females constituted 101 cases (64.7%) and males 47 cases (30.1%). Normal HPLC pattern was observed in 87 cases (55.8%), while 69 cases (44.2%) showed abnormal or variant patterns. The most frequent abnormality was sickle cell trait (34 cases, 21.8%), followed by beta-thalassemia trait (20 cases, 12.8%) and sickle cell disease/HbS homozygous pattern (9 cases, 5.8%). Rare patterns included HbS/beta-thalassemia, HbD trait, HbE trait, HbE/beta-thalassemia and possible alpha-thalassemia.

Conclusion: HPLC is an effective and reliable technique for screening and characterization of hemoglobinopathies. The present study highlights the occurrence of different hemoglobin variants in the island population and supports the importance of screening and genetic counseling programs.

Keywords: Hemoglobinopathy, HPLC, Sickle Cell Disease, Beta-Thalassemia Trait, HbS, HbE, HbD, Andaman and Nicobar Islands.

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Introduction

Hemoglobinopathies comprise a group of inherited disorders caused either by reduced synthesis of globin chains or by structural abnormalities in hemoglobin molecules. These disorders include thalassemias and variant hemoglobins such as HbS, HbD, and HbE. India carries a substantial burden of hemoglobinopathies because of ethnic diversity, endogamous practices, and population migration.[5]

β-thalassemia and sickle cell disorders are important causes of chronic anemia and transfusion dependency in India.[5] Early diagnosis is essential for patient management, antenatal counseling, carrier detection, and prevention of severe disease in offspring.[1]

High-Performance Liquid Chromatography (HPLC) has emerged as a rapid and reliable method for evaluation of hemoglobin disorders because of its accuracy, reproducibility, and rapid processing time.[2,3] HPLC allows quantitative estimation of hemoglobin fractions including HbA0, HbA2, HbF, and abnormal variants.[4,6] Several Indian studies have demonstrated the usefulness of HPLC in diagnosis and epidemiological evaluation of hemoglobinopathies.[7,8,9,10] However, published

literature from the Andaman and Nicobar Islands remains limited. Therefore, the present study was undertaken to assess the spectrum of hemoglobinopathies diagnosed by HPLC in a tertiary care centre in this region.

Aim and Objectives

Aim: To determine the spectrum of hemoglobinopathies diagnosed by HPLC at a tertiary care centre in the Andaman and Nicobar Islands.

Objectives

1. To determine the frequency of different HPLC patterns and hemoglobinopathies
2. to study age and sex distribution
3. to describe relevant HPLC patterns and hemoglobin fractions
4. to highlight the diagnostic utility of HPLC in routine hematology practice.

Materials and Methods

Study Design: Retrospective observational study.

Study Setting: Department of Pathology, ANIIMS & GB Pant Hospital, Port Blair, Andaman and Nicobar Islands.

Study Period: 1st April 2025 to 31st March 2026.

Sample Size: A total of 156 HPLC cases were included.

Inclusion Criteria: All patients undergoing HPLC testing during the study period and cases with complete HPLC records were included.

Exclusion Criteria: Incomplete records & Repeat samples were excluded from this study.

Variables Studied: Age, sex, clinical indication, hemoglobin concentration, HbA0, HbA2, HbF, HbS, HbD, HbE, final HPLC diagnosis and verification remarks.

HPLC Analysis: Hemoglobin fraction analysis was performed using a Lifotronic 8 HPLC analyzer in the Department of Pathology. The analyzer separates hemoglobin fractions and provides quantitative results for HbA0, HbA2, HbF and variant peaks such as HbS, HbD and HbE. Results were interpreted with clinical details, hemoglobin values and HPLC fraction patterns. Borderline or discordant cases were marked for repeat testing.

Statistical Analysis: Data were entered into Microsoft Excel and analyzed using descriptive statistics including frequencies and percentages. Continuous variables were summarized as mean ± standard deviation and median where applicable. Graphs were generated for diagnosis distribution, age distribution, sex distribution and abnormal HPLC patterns.

Results

A total of 156 HPLC records were analysed. Age was recorded in 145 cases and was not recorded in 11 cases. The mean age was 23.64 ± 15.42 years. Hemoglobin value was available in 153 cases, with a mean hemoglobin of 8.99 ± 2.26 g/dL. Females formed the majority of the study population.

Table 1: Demographic characteristics of the study population

Characteristic	Value
Total cases	156
Age recorded	145 (92.9%)
Age not recorded	11 (7.1%)
Mean age ± SD	23.64 ± 15.42 years
Median age	23 years
Hemoglobin recorded	153 (98.1%)
Mean Hb ± SD	8.99 ± 2.26 g/dL
Female	101 (64.7%)
Male	47 (30.1%)
Sex not recorded	8 (5.1%)

Table 2. Spectrum of HPLC diagnoses

Diagnosis Category	Number	Percentage
Normal HPLC pattern	87	55.8
Sickle cell trait / HbS trait	34	21.8
Beta-thalassemia trait / possible	20	12.8
Sickle cell disease / HbS homozygous	9	5.8
HbS/beta-thalassemia pattern	2	1.3
HbD trait	1	0.6
HbE trait	1	0.6
HbE/beta-thalassemia	1	0.6
Possible alpha-thalassemia	1	0.6

Table 3: Age distribution

Age Group	Number	Percentage
<1 year	5	3.2
1-5 years	18	11.5
6-10 years	6	3.8
11-20 years	35	22.4
21-40 years	60	38.5
41-60 years	16	10.3
>60 years	5	3.2
Not recorded	11	7.1

Table 4: Sex distribution

Sex	Number	Percentage
Female	101	64.7
Male	47	30.1
Not recorded	8	5.1

Table 5: Diagnosis category by sex

Diagnosis Category	Female	Male	Not recorded	Total
Normal HPLC pattern	56	26	5	87
Sickle cell trait / HbS trait	24	9	1	34
Beta-thalassemia trait / possible	12	7	1	20
Sickle cell disease / HbS homozygous	5	3	1	9
HbS/beta-thalassemia pattern	1	1	0	2
HbD trait	1	0	0	1
HbE trait	0	1	0	1
HbE/beta-thalassemia	1	0	0	1
Possible alpha-thalassemia	1	0	0	1

Table 6: Hemoglobin values by diagnosis category

Diagnosis Category	n with Available Hb Data	Mean Hb	SD	Median Hb	Range
Normal HPLC pattern	84	8.73	2.34	8.5	3.3-14.7
Sickle cell trait / HbS trait	34	10.09	1.68	10.25	6.2-13.8
Beta-thalassemia trait / possible	20	8.93	2.28	9.25	4.3-13.1
Sickle cell disease / HbS homozygous	9	8.09	1.43	7.6	6.8-10.3
HbS/beta-thalassemia pattern	2	7.65	2.05	7.65	6.2-9.1
HbD trait	1	10.1	-	10.1	10.1
HbE trait	1	13.3	-	13.3	13.3
HbE/beta-thalassemia	1	5.1	-	5.1	5.1
Possible alpha-thalassemia	1	5.0	-	5.0	5.0

Normal HPLC pattern was the commonest finding, noted in 87 cases (55.8%). Among abnormal patterns, sickle cell trait was most frequent, followed by beta-thalassemia trait and sickle cell disease. Sickle cell-related disorders, including sickle cell trait, sickle cell disease and HbS/beta-thalassemia pattern, together accounted for 45 cases (28.8% of all cases).

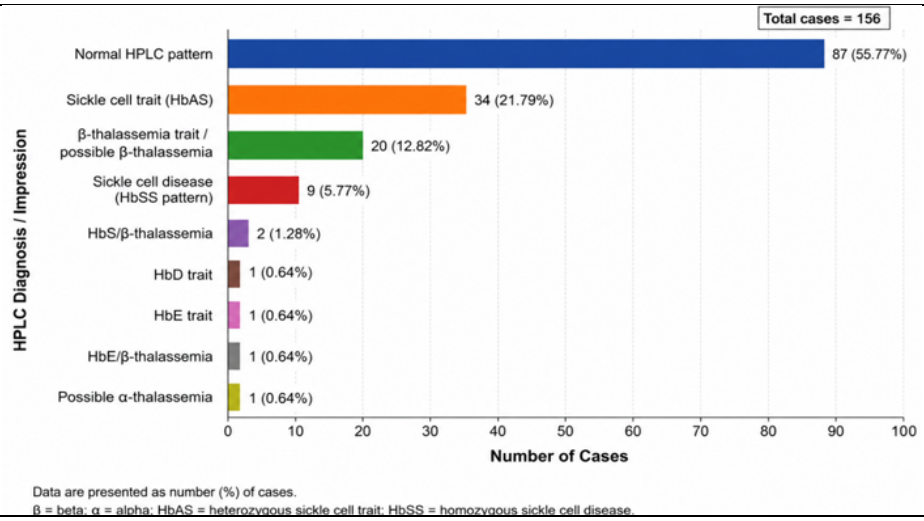

Figure 1: Spectrum of Hemoglobinopathy Diagnoses by HPLC (n=156)

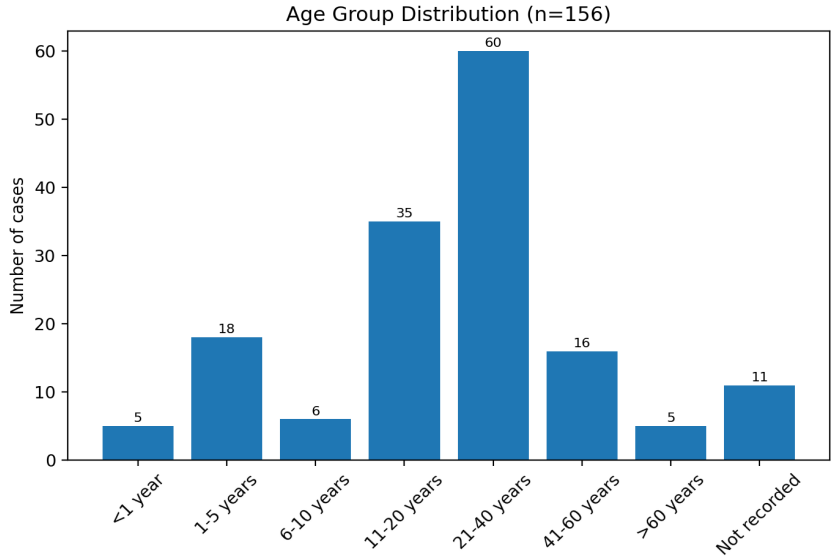

Figure 2: Age group distribution of cases

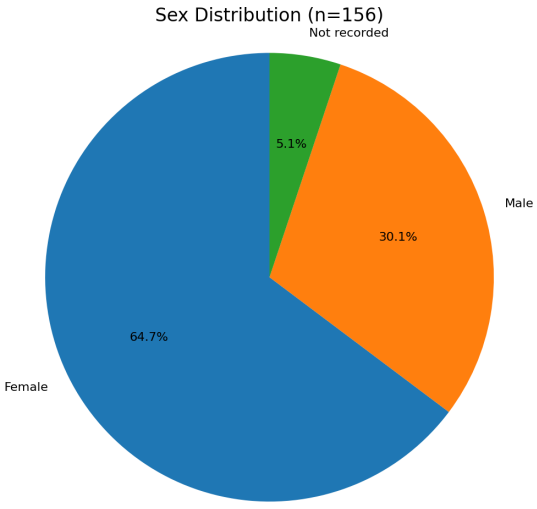

Figure 3: Sex distribution of cases

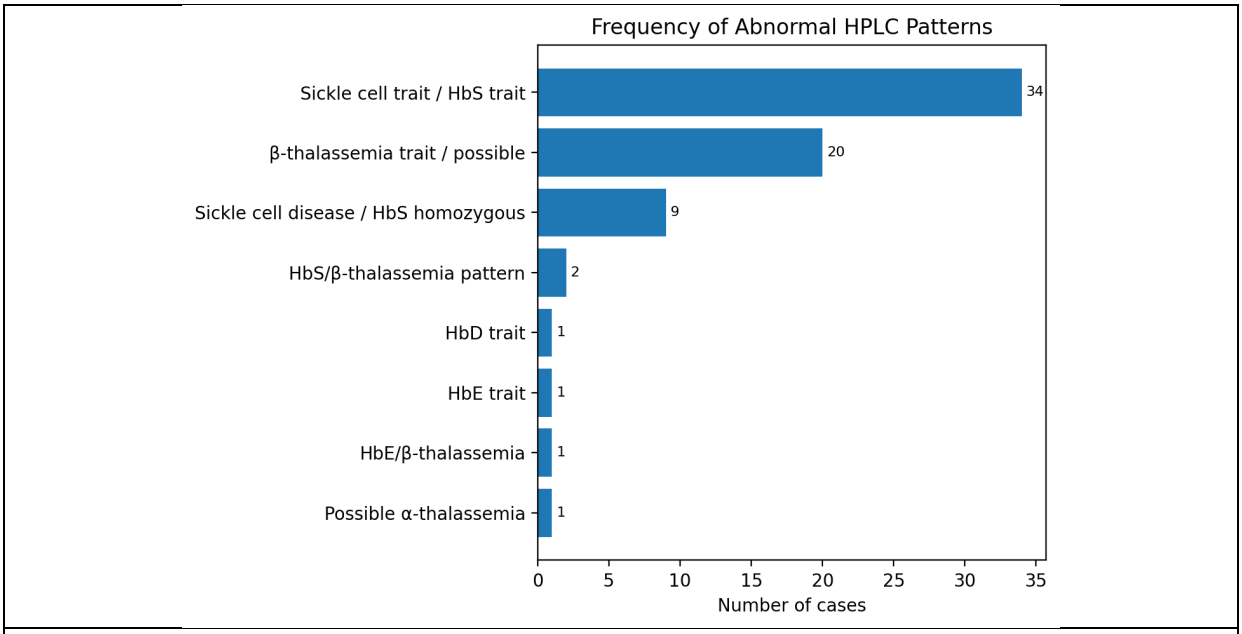

Figure 4: Frequency of abnormal HPLC patterns

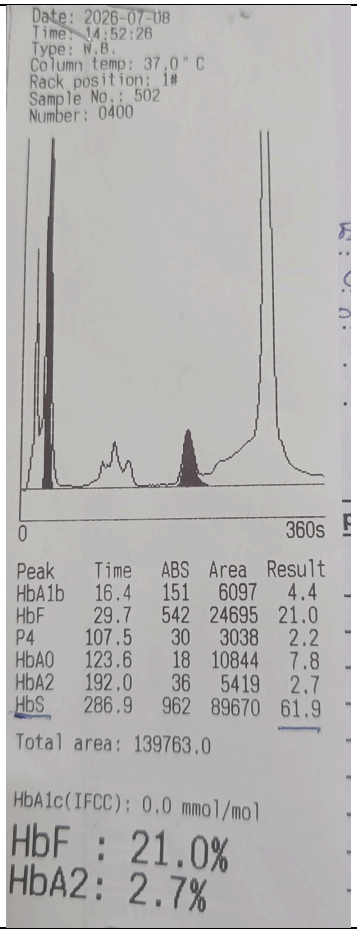

Figure 5: Representative HPLC chromatogram showing HbS homozygous/sickle cell disease pattern: HbS 61.9%, HbF 21.0%, HbA0 7.8% and HbA2 2.7%

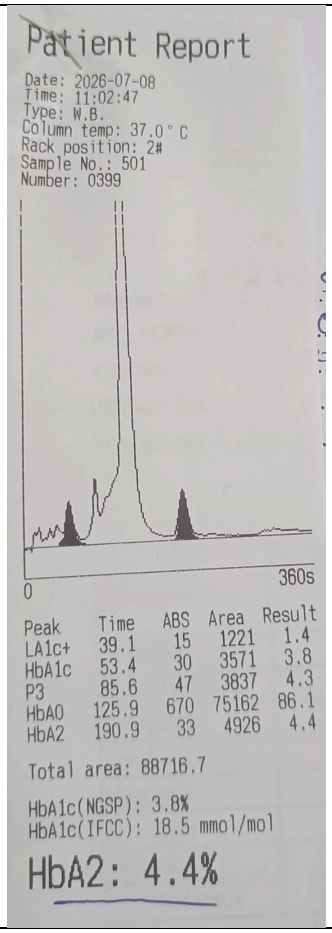

Figure 6: Representative HPLC chromatogram showing beta-thalassemia trait pattern with HbA2 4.4%

Discussion

The present retrospective study evaluated 156 HPLC cases over a one-year period and demonstrated that

sickle cell trait was the most frequently encountered abnormal hemoglobin variant. Similar observations have been reported in several Indian studies evaluating the spectrum of hemoglobinopathies using HPLC, although the relative frequencies of individual variants vary according to regional ethnic distribution and population genetics. [7,8,9] The predominance of sickle cell trait in the present study may reflect the heterogeneous population of the Andaman and Nicobar Islands, where migration from different regions of India has contributed to considerable genetic diversity.

β-thalassemia trait constituted the second most common abnormal HPLC pattern. Elevated HbA_2 levels remained the most reliable laboratory marker for its diagnosis, consistent with previous studies demonstrating the high sensitivity and specificity of HPLC for carrier detection. [2,4,3] Early identification of β-thalassemia carriers is of considerable public health importance, as timely genetic counseling and premarital or antenatal screening can substantially reduce the birth of children affected with transfusion-dependent β-thalassemia major.

A considerable proportion of cases in the present study were detected during antenatal evaluation and family screening rather than because of clinical symptoms, highlighting the value of opportunistic screening programs in identifying asymptomatic carriers. Similar observations have been emphasized by Colah et al., who advocated population screening and genetic counseling as effective strategies for reducing the burden of inherited hemoglobin disorders in India.[5]

In addition to heterozygous disorders, homozygous sickle cell disease and compound hemoglobinopathies, including HbS/β-thalassemia, were also identified. These cases generally demonstrated higher HbF levels, which may influence disease severity and clinical presentation. Recognition of such complex hemoglobinopathies is important because they require appropriate clinical follow-up, counseling, and individualized management.

The present study further confirms the usefulness of HPLC as a rapid, reliable, and cost-effective diagnostic technique for routine screening and characterization of hemoglobin variants. Besides accurately quantifying HbA, HbA_2, HbF, and abnormal hemoglobin fractions, HPLC enables simultaneous detection of multiple hemoglobin variants with excellent reproducibility. Similar advantages have been reported by Clarke et al., Joutovsky et al., and Baig et al., supporting the routine use of HPLC in diagnostic laboratories. [2,3,6]

The findings of this study also provide valuable baseline epidemiological data on the spectrum of hemoglobinopathies in the Andaman and Nicobar Islands, a geographically isolated region with a genetically diverse population and limited published literature. These data may assist clinicians, public health authorities, and policymakers in planning targeted screening programs, strengthening carrier detection strategies, and expanding genetic counseling services in the region.

Limitations

This was a retrospective laboratory record-based study. Clinical details and CBC parameters were not uniformly available for all cases. Some entries required verification because of incomplete or borderline values. HPLC interpretation may be affected by recent transfusion, age of the patient, iron deficiency and coexisting variants. Molecular confirmation was not available for all cases.

Conclusion

High-performance liquid chromatography (HPLC) is a rapid, reliable, and effective diagnostic tool for the screening and characterization of hemoglobinopathies. In this retrospective study of 156 cases from a tertiary care centre in the Andaman and Nicobar Islands, a normal HPLC pattern was the most common overall finding, while sickle cell trait was the most frequent abnormal hemoglobin variant, followed by β-thalassemia trait and sickle cell disease. The study provides valuable baseline data on the regional spectrum of hemoglobinopathies and highlights the importance of HPLC in the early detection of carriers and affected individuals. These findings support the implementation of targeted population screening, antenatal and family screening, genetic counseling, and timely clinical intervention to reduce the burden of inherited hemoglobin disorders in the region.

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