

Spectrum and Prevalence of Hemoglobinopathies in a Tertiary Care Hospital: A Retrospective Study Based on Hematological Parameters and High-Performance Liquid Chromatography (HPLC)

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Abstract

Hemoglobinopathies constitute a major group of inherited red cell disorders resulting from qualitative or quantitative abnormalities of globin-chain synthesis. Thalassemias and structural hemoglobin variants such as HbS, HbE and HbD are important causes of anemia in India, with considerable geographical variation in their distribution. High-performance liquid chromatography (HPLC) provides simultaneous separation and quantification of major hemoglobin fractions and is widely used for laboratory evaluation of hemoglobin disorders.

Aim: (1) To determine the spectrum and frequency of hemoglobinopathies detected by HPLC in patients evaluated at a tertiary care hospital. (2) To correlate the HPLC findings with hematological parameters, age and sex distribution.

Materials and Methods: A retrospective observational study was conducted in the Department of Pathology, NAMO Hospital, Silvassa, from January 2026 to August 2026. A total of 240 patients evaluated for anemia, suspected hemoglobinopathy, abnormal peripheral smear findings, family history of hemoglobinopathy, antenatal/preconception screening or other relevant indications were included. Complete blood count and red-cell indices were obtained using an automated five-part hematology analyzer. Hemoglobin fractions were analyzed using a Bio-Rad D-10 HPLC analyzer. HPLC patterns were interpreted on the basis of retention time and percentage of individual fractions. Patients with recent blood transfusion, technically inadequate samples or invalid HPLC chromatograms were excluded.

Results: Among the 240 patients evaluated, 135 (56.25%) showed an HPLC pattern consistent with a hemoglobinopathy. The most frequently identified abnormality was HbS heterozygosity (sickle cell trait), observed in 43 cases (31.85% of hemoglobinopathy cases), followed by HbS homozygous pattern/sickle cell disease in 38 cases (28.15%) and beta-thalassemia trait in 29 cases (21.48%). Other findings included borderline HbA2 in 15 cases (11.11%), beta-thalassemia major in 5 cases (3.70%), HbE heterozygosity in 2 cases (1.48%), HbD Punjab heterozygosity in 2 cases (1.48%) and HbE homozygosity in 1 case (0.74%). The study population showed a female predominance (177; 73.75%), and the largest age group was 21–30 years (135; 56.25%).

Conclusion: A substantial proportion of patients evaluated in this tertiary-care setting demonstrated hemoglobinopathy-associated HPLC patterns, with sickling disorders constituting the predominant abnormalities. HPLC, when interpreted alongside CBC parameters and peripheral blood morphology, provides an effective laboratory approach for screening and characterization of common hemoglobin disorders. Regional screening, particularly among young adults and individuals undergoing antenatal or preconception evaluation, may facilitate early recognition of carrier states and affected individuals.

Keywords: Hemoglobinopathy; HPLC; Sickle Cell Trait; Sickle Cell Disease; Beta-Thalassemia; Anemia.

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Introduction

Hemoglobinopathies are a diverse group of inherited disorders caused by qualitative or quantitative defects in the synthesis of hemoglobin (Hb) chains. Among these, thalassemias and structural variants such as HbS, HbE, and $\delta\beta$ -thalassemia trait are particularly prevalent in India and represent a significant public health burden due to high carrier frequencies and limited awareness among the general population.[1]

Haemoglobinopathies may result from mutation of a beta globin gene, in which case there is a variant form of haemoglobin A, or from mutation of an alpha globin gene, in which case there are variant forms of haemoglobins F, A and A2. Similarly, mutations of gamma and delta genes result in mutant forms of haemoglobin F and haemoglobin A2, respectively. [2]

Thalassemias are a group of hemolytic anemias which result from an inherited abnormality of globin production. Clinical and hematologic features of these cases are due to reduced hemoglobin production and accumulation of alpha or beta globin chains. [3] Hb D (HbD Punjab) is primarily seen in the northwestern parts of the country, while sickle cell trait (SCT) and thalassemia disease are more frequent in central and tribal belts. These hemoglobinopathies may range in clinical severity from asymptomatic carrier states to life-threatening anemias requiring lifelong transfusion support, particularly in conditions like thalassemia major (TM).[1]

The diagnosis of disorders of haemoglobin chain synthesis usually requires a combination of techniques. A complete blood count (CBC) and a blood film examination are usually indicated whenever an abnormality of globin chain synthesis is suspected. Haemoglobin electrophoresis is still the most common technique for the initial detection and characterization of a variant haemoglobin, although high performance liquid chromatography (HPLC) is increasingly taking its place. HPLC can be used not only for the detection, provisional identification and quantification of variant haemoglobins, but also for the quantification of haemoglobins A, A2 and F. [2]

Despite advancements in diagnostic modalities, the implementation of comprehensive community screening and regional epidemiological mapping remain insufficient. There is a pressing need to study the spectrum and prevalence of hemoglobinopathies across different regions to inform local healthcare policies and guide targeted interventions.[4] The present study aimed to assess the spectrum of hemoglobinopathies in a tertiary care population, correlate HPLC findings with hematological indices,

and analyze age and gender distribution of affected individuals.

Aim: To study the spectrum and frequency of hemoglobinopathies in patients evaluated at a tertiary-care hospital using hematological parameters and high-performance liquid chromatography.

Objectives

1. To determine the frequency and spectrum of hemoglobinopathies detected by HPLC.
2. To study the age and sex distribution of patients with hemoglobinopathies.
3. To correlate HPLC findings with hemoglobin and red-cell indices.
4. To evaluate the utility of HPLC in the laboratory diagnosis of common hemoglobin disorders.
5. To identify the relative contribution of sickling disorders, thalassemias and other structural hemoglobin variants in the study population.

Material and Method

Study Design: A retrospective observational study.

Study Setting: study conducted in Department of pathology, NAMO Hospital, Silvassa.

Study Duration: January 2026 to august 2026.

Study Population and Size: A total of 240 anemic patients were included, whose samples were received from different outpatient department, wards of the hospital and regional primary health center. Informed consent was obtained from all participants before inclusion in the study.

Inclusion Criteria

1. Patients of all age groups and both sexes.
2. Patients referred for evaluation of:
 - Anemia
 - Suspected hemoglobinopathy
 - Abnormal peripheral smear
 - Family history of hemoglobinopathy
 - Antenatal/preconception screening
 - Preoperative or routine hemoglobinopathy screening, where applicable.
3. Samples having adequate clinical and laboratory information for analysis.
4. Samples with a technically satisfactory HPLC chromatogram allowing identification/interpretation of hemoglobin fractions.

Exclusion Criteria

- Repeat samples from the same patient during the same study period.
- Samples with inadequate quantity, clotting, leakage, or improper collection/storage that prevents reliable analysis.

- Poor-quality or technically invalid HPLC chromatograms.
- Samples with incomplete patient identification or insufficient demographic information.
- Patients who received recent blood transfusion within previous 3 months.

Methodology

Hb and red blood cell (RBC) indices were measured on automated five-part differential cell counter (Siemens ADVIA 2120i). The results of Hb, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), RBC count, and red cell distribution width (RDW-CV) were correlated with peripheral blood examination. Sickling test was performed whenever indicated. All these samples were analyzed for Hb disorders by HPLC using a Bio-Rad D-10 analyzer. Data on age, gender, HPLC findings, red cell indices (Hb, MCV, MCH, MCHC, RBC count, and RDW-CV), and Hb fractions (HbA, HbA₂, HbF, and variant Hbs) were collected and analyzed.

Procedure

- 5 mL of blood was drawn from each participant using venipuncture and collected in EDTA tubes.
- The samples were labeled, stored, and transported under standardized conditions to ensure sample integrity.
- Blood samples were first lysed using specific lysing agent or procedure.
- Hemolysates were then injected into the HPLC system.
- Separation of Hb variants was based on their differential retention times (RTs). The identification of Hb variants was made primarily based on RT windows and area percentage.
- Reference standards and controls were run alongside the samples to ensure the accuracy of the identified peaks.

Result

All the samples were analyzed using Bio-Rad D-10 HPLC analyzer. Various types of the hemoglobin abnormalities were detected on the basis of their retention time and area percentage by HPLC. The total acceptable area was 10-40 million. Values outside this range should not be reported and samples in case it was above the higher limit should be manually pre diluted and rerun.



Figure 1: Bio RAD D-10 analyser for HPLC analysis [HPLC- High performance liquid chromatography]

The total number of cases included in this study was 240. The age distribution of the patients showed a significant preponderance of young adults, particularly in the 21–30 years' age group, which accounted for 56.2% of the total cases (240 patients). The remaining cases were distributed across other age groups, with 23% (55 patients) in the 11–20 years' range, 13.3% (32 patients) in the 31–40 years

range, and the youngest group, 0–10 years, representing 7.5% (18 patients). These findings suggested that the condition under study predominantly affected young adults. [Table 1] The gender distribution of the patients revealed a female predominance, with 73.7% (177 patients) being female, while 26.2% (63 patients) were male. [Table 2]

Table 1: Age group wise distribution

Age Group	No. of Patients (%)
0-10 year	18 (7.5%)
11-20 years	55 (23%)
21-30 years	135 (56.2%)
31-40 years	32 (13.3%)
Total	240 (100%)

Table 2: Gender distribution

Gender	No. of Patients (%)
Female	177 (73.7%)
Male	63 (26.2%)
total	240 (100%)

The HPLC analysis revealed that among the 135 hemoglobinopathy cases, the majority had Sick cell trait (HbS heterozygous) = 43 cases, 31.9%, followed by sickle cell disease (HbS homozygous) = 38 cases, 28.1%. These results reflected a higher frequency of Sick cell related conditions in the study population, with HbS heterozygous being the most prevalent [Table 3].

Table 3: High-performance liquid chromatography interpretation with distribution of total number of cases percentage wise

HPLC interpretation	No. of cases (%)
Beta thalassemia trait	29 (21.5%)
Borderline A2	15 (11.1%)
Beta thalassemia Major	05 (3.70 %)
Hb S heterozygous (Sickle cell trait)	43 (31.9%)
Hb S homozygous (Sickle cell disease)	38 (28.1%)
Hb E heterozygous	02 (1.5%)
Hb E Homozygous	01 (0.74%)
Hb D Punjab Heterozygous	02 (1.5%)
Total	135 cases (100%)

The hematological parameters observed in patients with hemoglobinopathies are summarized as follows. [Table 4]:

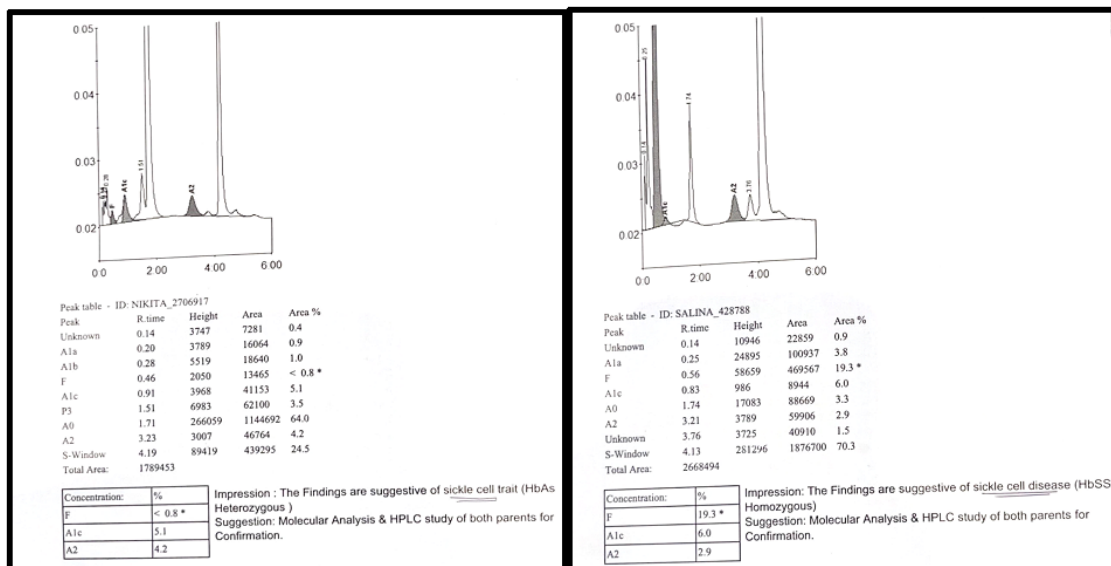
Table 4: RBCs Parameters in various Hemoglobinopathies

Diagnosis	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)	RBC ($\times 10^6/\mu\text{l}$)	RDW-CV (%)
Beta thalassemia trait	10-12 g/dl	65-80 fl	20-25 pg	29-31%	4.5-5.5	12-14 %
Borderline A2	11-12 g/dl	70-80 fl	21-24 pg	30-32 %	4.5-5.5	12-14 %
Beta thalassemia Major	3-8 g/dl	50-70 fl	12-20 pg	30-32%	2.0- 3.0	18-20 %
Hb S heterozygous (Sickle cell trait)	10-13 g/dl	80-85 fl	24-26 pg	30-32 %	4.0- 4.5	12-14 %
Hb S homozygous (Sickle cell disease)	4-8 g/dl	70-80 fl	20-24 pg	30-31 %	2.5-3.5	20-24%
Hb E heterozygous	8.5-9.5 g/dl	75-80 fl	20-24 pg	31-32 %	3.5-4.5	16-18 %
Hb E Homozygous	10.5 g/dl	65 fl	21 pg	31 %	4.8	15.7 %
Hb D Punjab Heterozygous	11-12 g/dl	80-83 fl	23-25 pg	29-30 %	4.5-5.5	14-15 %

The Hb profiles determined by HPLC are summarized as follows: [table 5]

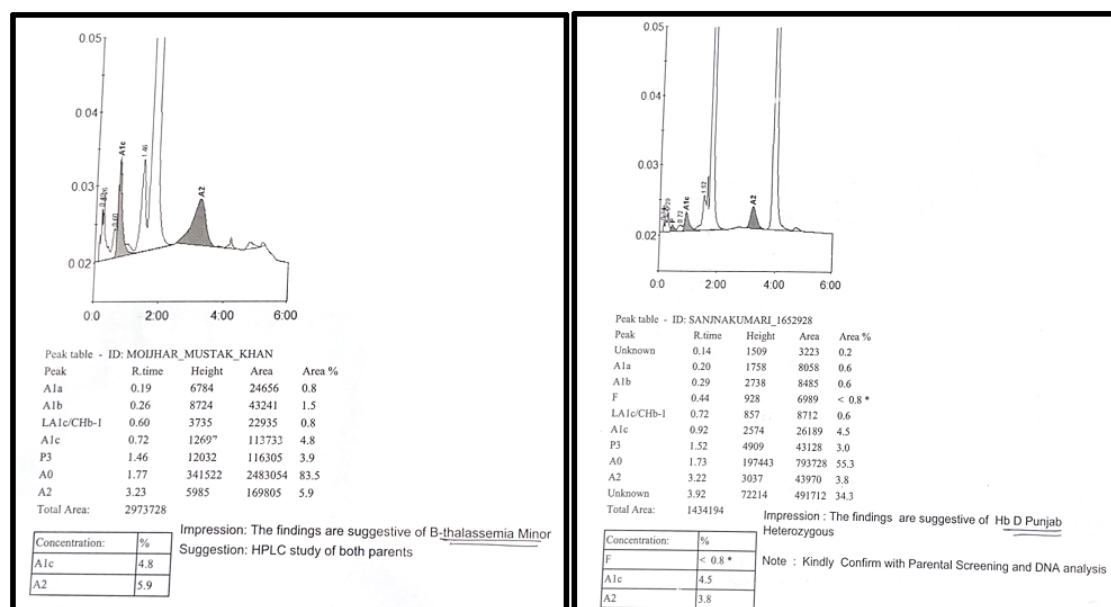
Table 5: Hemoglobin profile in each case obtained on high-performance liquid chromatography (mean $\pm$ standard deviation, range)

Diagnosis	Hb A0 (%)	Hb A2 (%)	Hb F (%)	other variants of Hb
Beta thalassemia trait	80-82 %	4-7 %	1-1.5 %	-
Borderline A2	80-82%	3.5-4%	0.5-1 %	-
Beta thalassemia Major	5-10 %	3-3.5 %	90-95 %	-
Hb S heterozygous (Sickle cell trait)	50-60 %	3-3.5 %	0.5-1 %	Hb S: 30-40 %
Hb S homozygous (Sickle cell disease)	5-10 %	2-3 %	10-15 %	Hb S: 70-80 %
Hb E heterozygous	60-65%	20-30 %	0.5-1 %	-
Hb E Homozygous	10%	80 %	1%	-
Hb D Punjab Heterozygous	50-60 %	3-3.8 %	0.5-1 %	D window : 30-40 %



(A) Sickle cell trait

(B) Sickle cell disease



(C) Beta thalassemia minor

(D) Hb D Punjab

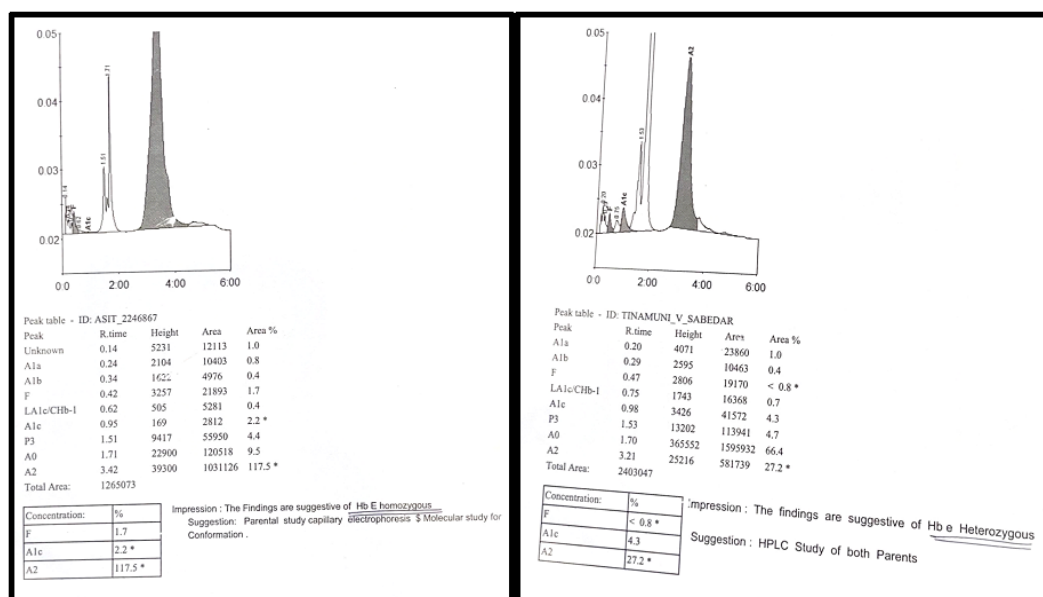


Figure 2: High-performance liquid chromatography chromatograms of common hemoglobinopathies with retention times and peak distribution

Discussion

Hemoglobinopathies are one of the major public health problems in the world including in India, which possesses a large burden on the health and financial resources of the country affected. As hemoglobinopathies should be a geographical variation, it is important to know about the actual prevalence of this lifelong disorder by conducting various regional studies. The early precise and accurate diagnosis of these conditions is also very important because it prevents the occurrence of clinically sense hemoglobinopathies and thus lessens the economic and psychological burden of this lifelong health problem.[7]

Most of the centers in India now use HPLC as a standard method for diagnosing various hemoglobin disorders. This is because, the conventional technique like alkaline and acid electrophoresis for hemoglobin variants, HbA2 quantification by ion-exchange column chromatography, and HbF quantification by alkali denaturation or radial immunodiffusion for thalassemia are time-consuming and labor-intensive and at a times are unable to detect many Hb variants having similar electrophoretic mobility. The conventional methods are further incapable of detecting multiple Hb fractions in a single-step procedure. Thus, the identification of Hb variants by these conventional techniques is often speculated.

Cation exchange HPLC is accepted as the gold standard for screening of various hemoglobinopathies because it accurately quantifies most of the commonly found Hb variants except some rare variants which get co-eluted with other normal and Hb variants having similar retention times. In such cases, further molecular studies are required for their definitive diagnosis.

In our study, we used Bio-Rad D-10 fully automated analyzer for HPLC analysis of various hemoglobinopathies. It detected most of the commonly found hemoglobinopathies very accurately, precisely, and rapidly as compared to the conventional techniques.

In this study of 240 patients evaluated using HPLC, hemoglobinopathies were diagnosed in 135 (56.2%) cases, with Sickle cell trait being the most prevalent (43 cases, 31.9%), followed by sickle cell disease (38 cases, 28.1%) and beta thalassemia trait (29 cases, 21.5%). These findings underscore the regional burden of Sickle cell syndromes and highlight the utility of HPLC in their accurate diagnosis.

Comparison of Age and Gender distribution: In this study of 240 patients, a female predominance was observed, with 73.7% (177 patients) female. Similar trend have been reported by Yadav et al. [1] and Meshram et al. [5] emphasizing increased screening in reproductive-age women.

The majority of patients (56.2%) were in the 21–30 years' age group, consistent with the reproductive-age population targeted for premarital and antenatal screening. This observation aligns with Yadav et al. [1] and Meshram et al. [5] who also reported a peak incidence of hemoglobinopathies in young adults, highlighting the importance of early detection in this age bracket to prevent transmission to offspring.

Prevalence and spectrum of hemoglobinopathies: Among the 135 (56.2%) hemoglobinopathy cases, Sickle cell trait was the most common (43 cases, 31.9%). Yadav et al. [1], Chandrashekar et al. [6], Meshram et al. [5] who documented BTT as the most common hemoglobinopathy in North India.

These hematological and HPLC profiles demonstrate the diagnostic accuracy of HPLC in detecting both common and rare hemoglobinopathies, including carrier states that may be missed on routine CBC alone. Early detection allows genetic counseling and preventive interventions, particularly in high-risk populations.

Limitations

1. The study population consisted of patients selected for anemia, suspected hemoglobinopathy or screening indications, resulting in potential referral bias.
2. HPLC-based classification does not establish the underlying molecular genotype in every case.
3. Confirmatory molecular testing was not reported in the available dataset.
4. Information regarding iron status, ferritin, transfusion history beyond the exclusion criterion, clinical severity and treatment history was not available for all cases.
5. Borderline HbA2 findings require clinical and hematological correlation and may require additional investigations.

Conclusion

This study confirms the high prevalence of sickle cell trait in Silvassa, Dadra Nagar Haveli particularly among young adult females. HPLC remains a reliable and precise diagnostic modality for both symptomatic and carrier states. Targeted screening programs, especially antenatal and premarital, are essential for early detection, genetic counseling, and prevention of severe hemoglobinopathies such as sickle cell disease. Incorporating routine HPLC-based screening into regional healthcare programs could significantly reduce the burden of hemoglobinopathies.

Declarations

Consent: The source manuscript states that informed consent was obtained from participants. This should be retained only if it is consistent with the actual institutional protocol for the retrospective study.

Data Availability: The data generated or analyzed during the present study should be made available according to institutional policy and journal requirements.

Author Contributions: To be completed according to the actual contribution of each author.

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