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# SPECTRUM AND DISTRIBUTION OF HEMOGLOBIN VARIANTS BY CATION-EXCHANGE HPLC: INSIGHTS FROM A TERTIARY CARE CENTER IN SOUTH GUJARAT

Hematology

**Dr. Pinal C. Shah** M.D Pathology, Associate Professor, Department Of Pathology, Government Medical College, VNSGU, Surat, Gujarat, India

**Dr. Kajal C. Tandel*** M.D Pathology, Senior Resident, Department Of Pathology, Government Medical College, VNSGU, Surat, Gujarat, India *Corresponding Author

**Dr. Angela A. Bhalja** M.D Pathology, 3rd Year Resident, Department Of Pathology, Government Medical College, VNSGU, Surat, Gujarat, India

**Dr. Mayur A. Jarag** M.D Pathology, Associate Professor, Department Of Pathology, Government Medical College, VNSGU, Surat, Gujarat, India

**Dr. Pooja M. Valiya** M.D Pathology, 2nd Year Resident, Department Of Pathology, Government Medical College, VNSGU, Surat, Gujarat, India

## ABSTRACT

**Introduction:** Hemoglobinopathies are genetic disorders affecting the structure or production of hemoglobin in red blood cells. The most widely used method for identifying abnormal hemoglobin variants is cation exchange high-performance liquid chromatography (CE-HPLC). **Aim:** To estimate quantitatively different hemoglobin variants using Bio-Rad Variant II HPLC and to identify the spectrum of abnormal variants in South Gujarat, India. **Materials & Methods:** A descriptive cross-sectional observational study was conducted between January–December 2022 at a tertiary health-care center, South Gujarat. All solubility-positive samples from indoor/outdoor patients and health centers were included. A total of 20,895 samples were screened; 4,794 underwent HPLC analysis. Percentages of HbA, $HbA_2$, HbF, HbS, and other variants were calculated. **Results:** Of 20,895 screened samples, 4,794 underwent HPLC, and 3,611 (75.3%) showed abnormal variants. The most common was sickle cell trait (81.0%), followed by sickle cell disease (18.0%). Rare variants included β-thalassemia trait (0.67%), β-thalassemia major (0.05%), HbS/β-thalassemia double heterozygotes (0.13%), and HbD Punjab trait (0.02) **Conclusion:** CE-HPLC is a simple, rapid, and reliable method for identifying hemoglobin variants. The study demonstrates high prevalence of sickle cell disorders in South Gujarat, with thalassemia as an additional burden. Early screening, counseling, and public health integration through programs like the National Sickle Cell Elimination Programme (2023–2030) are critical to reducing the societal impact.

## KEYWORDS

Hemoglobinopathies, Sickle Cell Disease, High Performance Liquid Chromatography

## INTRODUCTION

Hemoglobinopathies are the most common single-gene disorders worldwide and represent a major global health challenge [1]. The World Health Organization (WHO) estimates that over 300,000 infants are born annually with severe hemoglobinopathies, predominantly sickle cell disease and thalassemia syndromes [2]. In India alone, there are more than 45 million carriers, and approximately 15,000 infants are born each year with clinically significant hemoglobinopathies [3,4]. The cumulative gene frequency in India is around 4.2%, but this varies markedly across different regions and communities [5].

The clinical spectrum of these disorders ranges from silent carriers and asymptomatic traits to severe, transfusion-dependent conditions such as β-thalassemia major and sickle cell anemia, which impose significant morbidity, mortality, and economic burden [6,7]. Due to consanguinity, caste-based endogamy, and regional founder effects, some tribal and non-tribal communities in India exhibit a disproportionately high prevalence, making hemoglobinopathies a major public health concern [8,9]. The Government of India has therefore prioritized their control through initiatives like the National Thalassemia Control Program and the recently launched National Sickle Cell Elimination Programme (2023–2030) [10,11].

Accurate diagnosis of hemoglobinopathies is critical for patient management, genetic counseling, and public health strategies. Traditional methods such as hemoglobin electrophoresis, sickling tests, and solubility tests have diagnostic value but are limited by lower sensitivity and inability to precisely quantify hemoglobin fractions [12]. In contrast, cation-exchange high-performance liquid chromatography (CE-HPLC) has emerged as the gold-standard technique for screening, detection, and quantification of hemoglobin variants. CE-HPLC offers automated internal sample preparation, superior resolution, short assay time, reproducibility, and precise quantification of fractions such as HbA, $HbA_2$, HbF, HbS, HbD, and others [13,14].

Despite high prevalence, data on the spectrum of hemoglobinopathies in South Gujarat remain limited, necessitating updated region-specific studies. The present study was undertaken with the objective of identifying and analyzing the distribution patterns of abnormal hemoglobin variants using CE-HPLC in a tertiary care center in South Gujarat, India.

## MATERIALS AND METHODS

The present study was conducted at the Sickle Cell Laboratory, Department of Pathology, tertiary heath care center south Gujrat, India. It was designed as a cross-sectional observational study carried out between January 2022 and December 2022.

### Study Population

All solubility-positive samples received from indoor and outdoor clinical departments, cases with a positive family history or abnormal peripheral smear findings, and samples received from health care centers were included. Patients with a history of blood transfusion within the last three months were excluded from the study.

### Sample Collection and Processing

Two milliliters of venous blood was collected in EDTA vacutainers. Complete blood counts, including RBC indices, peripheral smear examination, and reticulocyte count, were performed. A sickling solubility test was carried out on all samples, and solubility-positive samples were processed for HPLC.

### HPLC Procedure

Analysis was performed using the Bio-Rad Variant II HPLC system. $HbA_2$/F calibrators and both normal and abnormal controls were run before patient samples. The percentages of HbA, $HbA_2$, HbF, HbS, and other variants were estimated and classified according to retention time and chromatogram patterns.

### Data Analysis

The data were compiled Microsoft excel and analysis was done in tabular and graphical formats. Descriptive statistics were used for frequency distribution, and gender/age-wise stratification was applied.

**Table 1: Age Wise Distribution of Cases**

| Age | Beta thalassemia trait | | Thalassemia major | | Sickle cell trait | | Sickle cell disease | | S beta thalassemia heterozygous | | HbD | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male | Female |
| 0-20 | 2 | 6 | 1 | 1 | 338 | 671 | 175 | 139 | 1 | 0 | 0 | 0 |
| 21-40 | 5 | 11 | 0 | 0 | 304 | 1420 | 110 | 202 | 1 | 0 | 0 | 1 |
| 41-60 | 0 | 0 | 0 | 0 | 73 | 99 | 12 | 13 | 1 | 0 | 0 | 0 |
| 61-80 | 0 | 0 | 0 | 0 | 18 | 37 | 0 | 1 | 0 | 0 | 0 | 0 |
| >80 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 7 | 17 | 1 | 1 | 731 | 2195 | 297 | 355 | 3 | 1 | 0 | 1 |

## RESULTS

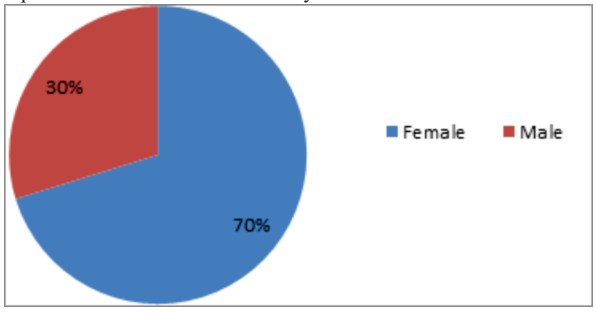
A total of 20,895 samples were screened for hemoglobinopathies during the study period, including 17,383 samples from indoor/outdoor patients and 3,512 samples from health care centers. Solubility testing was performed on all samples, and 4,794 samples underwent HPLC.

Out of the 4,794 HPLC samples, 718 (14.9%) showed normal hemoglobin patterns. Abnormal hemoglobin variants were detected in 3,611 cases (75.3%),

### Demographic Distribution

As shown in Table 1, the age of patients included in the study ranged from 1 month to 72 years, reflecting a wide spectrum across pediatric, adolescent, and adult populations. Abnormal hemoglobin variants were identified in a total of 3,611 cases, with a marked predominance among females (70%, n = 2,528) compared to males (30%, n = 1,083).

This corresponds to a male-to-female ratio of 1:2.4, indicating a higher representation of females in the study .




**Graph 1: Gender Wise Distribution of Hemoglobinopathies**

### Spectrum of Abnormal Hemoglobin Variants

The most common abnormality detected was sickle cell trait (n=2927, 81.0%), followed by sickle cell disease (n=652, 18.0%). The mean HbS level in sickle cell trait was 25.2% (range 20–40%), with hemoglobin levels between 9–11 g/dl and slightly reduced MCV. In sickle cell disease, the mean HbS was 76.1%. Solubility test was positive in all such cases.

β-thalassemia trait was detected in 24 cases (0.67%), characterized by elevated $HbA_2$ (5.1–6.8%) with low MCV, low MCH, and raised RBC count. Two cases (0.05%) of β-thalassemia major were noted: one 1-year-old male (HbF 77%, $HbA_2$ 14.3%) and one 4-year-old male (HbF 91.9%, $HbA_2$ 5.5%). Parental studies confirmed diagnosis in both.

Rare variants included HbD Punjab trait (n=1, HbD 32.9%) and HbS/β-thalassemia double heterozygotes (n=5), all showing elevated HbF levels with peaks in both S and D windows.

**Table 2: Distribution of Abnormal Hemoglobin Variants in South Gujarat**

| Abnormal hemoglobin variants | Number of patients | % |
|---|---|---|
| Sickle cell trait | 2927 | 81.0 |
| Sickle cell disease | 652 | 18.0 |
| Beta thalassemia trait | 24 | 0.67 |
| Thalassemia major | 2 | 0.05 |
| S-beta double heterozygous | 5 | 0.13 |
| hbD trait | 1 | 0.02 |
| Grand total | 3611 | 100 |

## DISCUSSION

India is an ethnically diverse country with marked regional variations, and this diversity is reflected in the distribution of hemoglobin variants across different communities. With increasing migration and inter-regional admixture, these variants are no longer confined to specific populations but are now detected across multiple states. The sickle cell gene is reported with higher frequency in Orissa, Assam, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, and Gujarat, whereas β-thalassemia is more commonly observed in Sindhi, Gujarati, Punjabi, and Bengali communities. These patterns highlight the need for both region-specific and pan-India strategies for screening, prevention, and management of hemoglobinopathies.

In the present study, 718 cases (14.9%) demonstrated normal hemoglobin patterns characterized by HbA as the predominant fraction, $HbA_2$ <3.3%, and HbF <0.8%. Interpretation of these findings requires caution, as confounding factors may alter $HbA_2$ levels. Vitamin $B_{12}$ deficiency can spuriously elevate $HbA_2$, whereas iron deficiency anemia may reduce $HbA_2$, thereby masking β-thalassemia trait. Such variability highlights the necessity of evaluating hematological indices and nutritional status in conjunction with HPLC findings to avoid diagnostic pitfalls.

The most common abnormality detected in our study was sickle cell trait (2927 cases, 81.0%), followed by sickle cell disease (652 cases, 18.0%). These results are in concordance with the studies of Saxena et al.(15) and Bhokare et al. (16),, who also reported sickle cell trait as the predominant abnormality in their study populations. The high prevalence of sickle disorders in this region underscores the substantial and persistent burden of this inherited condition across western and central India and directly supports the rationale for the recently launched National Sickle Cell Elimination Programme (NSCEP, 2023–2030), which aims to reduce morbidity and mortality through early detection, counseling, and integration of care into the public health system.

We identified 24 cases (0.67%) of β-thalassemia trait and 2 cases (0.05%) of β-thalassemia major. This finding contrasts with reports from northern India, where β-thalassemia trait predominates, as reported by Rao et al.(17), Sehgal et al.(18) (New Delhi), and Manger et al. (19) (North Gujarat). These regional differences may be be attributed to differences in founder mutations, the practice of endogamy in specific communities, and variable migration patterns across the regions. Despite these variations, β-thalassemia trait remains clinically important nationwide, as undetected carriers in a couple can result in the birth of a β-thalassemia major child when both partners are carriers.

Five cases of compound heterozygotes (HbS/β-thalassemia) were detected. The phenotypic expression in such individuals varies depending on the coinherited mutations, ranging from mild anemia to severe disease requiring transfusion. In our series, several patients had a history of transfusion and peripheral smear revealed microcytic hypochromic anemia. This underlines the need for integrating HPLC findings with morphological evaluation and, wherever feasible, family studies to accurately characterize hemoglobinopathy patterns.

Rare variants were also detected in our study, including one case of HbD Punjab trait. While our frequency was low, Manger et al(19). reported three cases from North Gujarat and Pant et al(20). reported 15 cases from New Delhi. HbD Punjab, though clinically silent in the heterozygous state, gains significance because of its potential to interact with HbS to produce HbSD disease, which may mimic sickle cell disease clinically.

Comparison with studies from Central India provides additional context. A cohort of 13,587 individuals screened in Central India reported sickle cell disease in 12% and homozygous β-thalassemia in 0.6%, demonstrating a substantial burden of SCD particularly among tribal populations (Singh MPSS et al., 2025(21) . A pan-India HPLC-based analysis revealed HbS trait as the most frequent variant nationally (~33%), with the highest prevalence reported from

Chhattisgarh (Colah et al., 2015) [22]. Additionally, a multi-center CE-HPLC study across India (65,779 cases) identified β-thalassemia trait as the most common abnormal variant, representing 11.21% of cases (Patel et al., 2017) [23]. A study from Karnataka by Putchen DD et al. reported 17.18% thalassemia, 6.42% other variants, and 1.08% double heterozygous states [24].

Compared to these data, South Gujarat shows clear predominance of sickle cell disorders, while β-thalassemia trait remains comparatively rare. These differences likely reflect regional ethnic composition, endogamy, and genetic drift, emphasizing the need for region-specific screening and preventive strategies.

High-performance liquid chromatography (HPLC) proved to be a rapid and reliable method for identifying and quantifying hemoglobin fractions. Its strengths include reproducibility, precision, and suitability for large-scale screening. However, limitations exist: HPLC may not reliably distinguish between certain variants and normal $HbA_2$, particularly in the presence of α- or β-thalassemia. Additionally, nutritional deficiencies may influence $HbA_2$ and HbF levels, complicating interpretation. In such cases, molecular analysis for definitive mutation detection is indispensable for accurate diagnosis and appropriate genetic counseling.

From a public health standpoint, the high prevalence of hemoglobinopathies in our study highlights the pressing need for comprehensive control measures. These should include:

- Community-based screening of high-risk populations,
- Antenatal and premarital counseling to minimize the risk of homozygous disease,
- Integration of nutritional assessment into hemoglobinopathy screening programs, and
- Establishment of molecular diagnostic facilities at regional centers for confirmatory testing.

Our findings resonate with the objectives of the National Health Mission's Thalassemia Control Program, which emphasizes early diagnosis, carrier detection, and awareness generation. Furthermore, the National Sickle Cell Elimination Programme (2023–2030), launched under India's tribal health mission, seeks to eliminate sickle cell disease as a public health problem by 2047 through universal newborn screening in high-prevalence areas, genetic counseling, and integration of services into primary healthcare. Together, these initiatives provide a comprehensive framework that, if effectively implemented, can substantially reduce the burden of hemoglobinopathies in India and improve patient outcomes.

## CONCLUSION

Hemoglobinopathies continue to exert a significant health burden in India, with particularly high prevalence in Gujarat. Early detection, prevention, and appropriate treatment remain essential to mitigate their impact. Cation-exchange HPLC has emerged as one of the most effective methodologies for screening and detection of hemoglobin variants, offering rapid, reproducible, and precise results. Its additional ability to quantify HbF and $HbA_2$, alongside variant identification, in a single highly automated system enhances its clinical utility. The advantages of internal sample preparation, superior resolution, short assay time, and accurate quantification make HPLC ideally suited for routine use in clinical laboratories. When interpreted in conjunction with hemogram findings and family studies, HPLC can detect the majority of hemoglobin variants prevalent in this region. However, a subset of inconclusive cases still require confirmatory genetic and molecular studies to establish a definitive diagnosis.

## REFERENCES


1. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001.
2. WHO. Sickle-cell anaemia: report by the Secretariat. Geneva: World Health Organization; 2006.
3. Colah RB, Gorakshakar AC, Nadkarni AH. Global burden, distribution and prevention of β-thalassemias and hemoglobin E disorders. Expert Rev Hematol. 2010.
4. Modell B, Darlison M. Global epidemiology of haemoglobin disorders. N Engl J Med. 2008.
5. Balgir RS. The burden of haemoglobinopathies in India and the challenges ahead. Curr Sci. 2000.
6. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010.
7. Colah R, Surve R, Sawant P, et al. HPLC studies in hemoglobinopathies. Indian J Pediatr. 1999.
8. Balgir RS. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J Assoc Physicians India. 1996.
9. Mohanty D, et al. Sickle cell disease in India. Curr Opin Hematol. 2003.
10. Ministry of Health & Family Welfare, Government of India. National Sickle Cell Elimination Programme (NSCEP). 2023.
11. National Health Mission. Guidelines for Prevention and Control of Hemoglobinopathies in India. 2016.
12. Bain BJ. Haemoglobinopathy Diagnosis. 2nd ed. Wiley-Blackwell; 2011.
13. Ou CN, Rognerud CL. Diagnosis of hemoglobinopathies: electrophoresis vs HPLC. Clin Chim Acta. 2001.
14. Colah RB, et al. High-performance liquid chromatography for rapid diagnosis of hemoglobinopathies. Indian J Hematol Blood Transfus. 2014.
15. Saxena S, Jain R. Study of abnormal haemoglobin variants using cation exchange high-performance liquid chromatography (HPLC) in paediatric population of Gujarat, India. Trop J Path Microbiol. 2019;5(11):856–62. doi:10.17511/jopm.2019.i11.04
16. Bhokare SB, Phulgirkar PP, Joshi AR, Bindu RS. Spectrum of hemoglobinopathies by high-performance liquid chromatography with special reference to role of $HbA_2$ levels at a tertiary care centre. Int J Res Med Sci. 2016;4(12):5269–76. doi:10.18203/2320-6012.ijrms20164193
17. Rao S, Kar R, Gupta SK, Chopra A, Saxena R. Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC and modulating effects of nutritional deficiency anaemias from North India. Indian J Med Res. 2010;132(11):513–9.
18. Sehgal S, Khan S, Jetley S, Alvi Y. Evaluation of abnormal hemoglobin variants and hemoglobinopathies on D-10 analyzer – An institutional experience from North India. Online J Health Allied Scs. 2022;21(2):10.
19. Manger U, Panchal N, Patel V, Patel M, Shrivastav A. Detection of hemoglobinopathies and hemoglobin variants through HPLC in Northern Gujarat: a study of 2500 cases. SSR Inst Int J Life Sci. 2024;10(4):6020–7.
20. Pant L, Kalita D, Singh S, Kudesia M, Mendiratta S, Mittal M, et al. Detection of abnormal hemoglobin variants by HPLC method: common problems with suggested solutions. ISRN Hematol. 2014;2014:257805. doi:10.1155/2014/257805. Retraction in: ISRN Hematol. 2015;2015:498230. PMID: 27351019; PMCID: PMC4897512
21. Singh MPSS, Kumar R, Patel P, Uikey R, Mun A, Shanmugam R. Hemoglobinopathies among patients referred to a single centre in Central India: an observational study. Indian J Clin Biochem. 2025;40(1):121–6. doi:10.1007/s12291-023-01151-2
22. Colah R, et al. Regional heterogeneity of the sickle cell trait and disease in India: An analysis of HPLC data from 25,297 cases. Indian J Med Res. 2015;141(5):507–15. PMID: 25721910.
23. Patel J, et al. Hemoglobinopathies detected by CE-HPLC in 65,779 cases: a multicenter study across India. Indian J Pathol Microbiol. 2017;60(1):68–73. PMID: 28217567.
24. Putchen DD, Kulkarni S, Nanjundarao SS, Bhat DG, Venkatachala PK, Prasad SR. Retrospective study on the distribution of hemoglobinopathies in Karnataka—A laboratory experience. Indian J Pathol Microbiol. 2024;67:585–91.