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# Prevalence and characteristics of hemoglobinopathies in a tertiary care hospital: A study utilizing high-performance liquid chromatography (HPLC)

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**Abstract**
**Introduction**: Hemoglobinopathies pose a growing global health concern, necessitating accurate and efficient diagnostic approaches. High-performance liquid chromatography (HPLC) stands out as a premier method for screening, detecting, and characterizing various hemoglobinopathies, offering a valuable tool for managing this expanding health burden.
**Materials and Methods:** The present study was carried out in Central Haematology Lab, Department of Pathology, B.J. Medical college, Ahmedabad from period of August 2023 to November 2023 with 581 cases evaluated with an aim to identify various hemoglobinopathies seen in Indian population by high-performance liquid chromatography.
**Results:** A comprehensive analysis of 581 cases revealed that 321 (55.2%) had abnormal hemoglobinopathies, including Beta thalassemia trait (75.1%), Sickle cell trait (9.9%), Double heterozygous for thalassemia and sickle cell (5.3%), Sickle cell disease (2.5%), Hb D (2.2%), Iron Deficiency Anemia (IDA) (1.9%), Hb E (1.6%), Thalassemia major (0.6%), Beta delta homozygous HPFH(Hereditary Persistence of Fetal Hemoglobin) (0.6%), and Thalassemia intermedia (0.3%), while 260 cases (44.8%) had normal chromatograms, with 3 samples requiring repeat testing, 7 cases reserved for further opinion, and the remaining cases showing no abnormalities.
**Conclusion:** This study demonstrates the effectiveness of HPLC in diagnosing hemoglobinopathies in a resource-constrained setting. The high prevalence of Beta thalassemia trait and disease underscores the need for widespread adoption of HPLC technology in regions like India, where early detection and management can significantly impact patient outcomes. Our findings highlight the importance of HPLC as a rapid, accurate, and essential tool in the diagnosis and management of hemoglobin disorders.

**Keywords:** Hemoglobinopathies, HPLC (High-Performance Liquid Chromatography), beta thalassemia, sickle cell disease, diagnostic screening

## Introduction
Hemoglobinopathies are the most common single-gene disorders worldwide, affecting approximately 5% of the global population as carriers of potentially pathological hemoglobin (Hb) genes [1]. The World Health Organization estimates that over 300,000 babies are born annually with hemoglobinopathies, resulting in significant morbidity and mortality [2]. These inherited disorders of hemoglobin synthesis cause significant mortality and morbidity, particularly in developing countries [5].
In India, the prevalence of thalassemia trait and sickle cell anemia varies between 3-17% and 1-44%, respectively, with higher frequencies in certain communities due to consanguinity and endogamy [3, 4]. The burden of hemoglobinopathies has increased due to global migration and consanguineous marriages [1]. Without therapy, thalassemia major patients often die from cardiomyopathy before age 20 [6].
High-performance liquid chromatography (HPLC) has emerged as a valuable tool for diagnosing hemoglobinopathies, offering rapid, reproducible, and precise results [7]. HPLC depends on the interchange of charged groups on the ion exchange material with charged groups on the hemoglobin molecule [8]. This technique allows for the quantification of normal and abnormal hemoglobin variants in each sample, using a very small amount of sample [9].
The importance of accurate diagnosis and management of hemoglobinopathies cannot be overstated. Early detection and intervention can significantly impact patient outcomes,

reducing morbidity and mortality [10]. In resource-constrained settings, HPLC offers a reliable and efficient diagnostic approach, enabling healthcare providers to effectively manage these disorders [11].

**Aims and Objective**

This study aims to evaluate the role of HPLC in detecting hemoglobinopathies in a tertiary care teaching hospital. By examining the prevalence and distribution of hemoglobinopathies in our patient population, we hope to contribute to the understanding of these disorders and inform strategies for their diagnosis and management.

**Materials and Methods**

**Study Design and Setting:** This present study was conducted at the Central Haematology Laboratory, Department of Pathology, B.J. Medical College, Ahmedabad, from August 2023 to November 2023.

**Sample Collection and Processing:** Whole blood samples were collected in vacuum tubes containing EDTA and stored at 2-8°C for up to 4 days or at room temperature (15-30°C) for up to 1 day. Demographic data, including age, sex, clinical history, family history, and history of blood transfusion, were recorded.

**CBC Analysis:** Complete Blood Counts (CBC) were performed using the Horiba HORIBA Penta XLR Automated Cell Counter, which measured White Blood Cell Count (WBC), Red Blood Cell Count (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), and Platelet Count.

**Peripheral Smear Examination:** Peripheral smears were made on glass slides, fixed with methanol, stained with Giemsa stain, and examined under a binocular microscope to evaluate Red Blood Cell Morphology, White Blood Cell Morphology, Platelet Morphology, and the presence of Inclusions or Abnormalities.

**HPLC Analysis:** HPLC was performed on the BIO-RAD D10TM system (BIO-RAD Laboratories, USA) using the principle of CE-HPLC through a cartridge, with a PolyCAT A column (4.6 x 200 mm) and a mobile phase of sodium phosphate buffer and acetonitrile, at a flow rate of 1.5 mL/min. Specific retention times were defined by the manufacturer for each hemoglobin variant, and a sickling test was performed using Na-Metabisulphite to confirm sickle cell disease when the S window was eluted in a sample.

**Inclusion Criteria**

1. All cases where HPLC was performed for suspected hemoglobinopathies
2. Detection of anisopoikilocytosis on peripheral smear
3. Detection of sickle-shaped cells on peripheral smear
4. Positive sickling solubility test
5. History of more than 5 blood transfusions in the absence of trauma or clinical morbidity

**Exclusion Criteria**

1. Rejected samples in the pre-analytical phase (e.g., inadequate, clotted samples)
2. Samples with incomplete demographic data
3. Samples with no HPLC results available.

**Ethics:** The study was approved by the Institutional Ethics Committee of B.J. Medical College, Ahmedabad.

**Quality Control:** Quality control measures were taken to ensure the accuracy and precision of the HPLC results. These measures included regular calibration of the HPLC system, use of quality control samples, and participation in external quality assurance programs.

**Data Analysis:** Descriptive statistics were used to analyze the data. The prevalence of hemoglobinopathies was calculated, and the results were presented in tables and figures.

**Table 1:** Proportion of different haemoglobins in normal individuals and in haemoglobin disorders according to surface area percentage

| Condition | HbA | HbF | HbA2 | HbS/C/D/E |
|---|---|---|---|---|
| Normal Adults | 95-98% | 0-2% | 2-4% | 0% |
| Sickle cell trait | 50-60% | 1-2% | 2-4% | 35-45% (HbS) |
| Sickle cell anemia | 5-20% | 0-5% | 1-3% | 80-95% (HbS) |
| β thalassemia trait | 80-90% | 1-5% | 5-15% | 0% |
| β thalassemia major | 0-20% | 80-100% | 0-5% | 0% |
| Iron deficiency anaemia | 90-95% | 2-5% | 0-2% | 0% |
| HbC Disease | 5-20% | 0-5% | 0% | 80-95% (HbC) |
| HbE Disease | 5-20% | 0-5% | 0% | 80-95% (HbE) |
| HbD Disease | 5-20% | 0-5% | 0% | 80-95% (HbD) |

The table shows the established ranges of different haemoglobin variants observed using the extended program. The printed chromatogram of HPLC displays all the haemoglobin fractions eluted, their retention times, peak areas, and values (%) of different haemoglobin components. If a peak elutes at a retention time that is not pre-defined, it is labelled as an unknown.

**Table 2:** Proportion of different haemoglobins in normal individuals according to retention time in minutes

| Peak Name | Retention Time (minutes) |
|---|---|
| HbA (Adult Hemoglobin) | 1.55-1.85 |
| HbA2 (Adult Hemoglobin Variant) | 0.38-0.58 |
| HbS (Sickle Hemoglobin) | 2.80-3.50 |
| HbF (Fetal Hemoglobin) | 4.02-4.30 |

Show proportion of different haemoglobins in normal individuals according to retention time in minutes

**Results**

This present study, conducted from August 2023 to November 2023 at the Department of Pathology, B.J. Medical College, Ahmedabad, analyzed 581 cases and found that 321 cases (55.3%) had abnormal hemoglobin patterns. Among the cases, 250 (43.03%) had normal chromatograms for age, 7 (1.20%) were reserved for opinion, and 3 (0.52%) required repeat samples. Additionally, 6 cases (1.03%) had iron deficiency anemia (IDA), characterized by high HbA2 levels.

**Table 3:** Distribution of hemoglobin variants

| Haemoglobin Pattern | Cases | (%) |
|---|---|---|
| Normal chromatogram for age | 250 | 43.03% |
| Reserved for opinion | 7 | 1.20% |
| Advice for repeat sample | 3 | 0.52% |
| Iron defiecency anemia | 6 | 1.03% |
| Sickle cell trait | 32 | 5.51% |
| Sickle cell disease | 8 | 1.38% |
| Thalessemia trait | 241 | 41.48% |
| Thalessemia intermedia | 1 | 0.17% |
| Thalassemia major | 2 | 0.34% |
| Beta delta homozygous HPFH (Hereditary Persistence of Fetal Hemoglobin) | 2 | 0.34% |
| Compound heterozygous sickle cell with beta thalessemia | 9 | 1.55% |
| Compound heterozygous sickle cell with alpha thalessemia | 8 | 1.38% |
| Hb E | 4 | 0.69% |
| Hb E trait with b thalessemia | 1 | 0.17% |
| Hb D (trait+homozygous+ with IDA) | 5+1+1=7 | 1.20% |
| Grand Total | 581 | |

The distribution of hemoglobin variants is presented in Table 3, which shows that beta thalassemia trait was the most common variant, occurring in 41.48% of cases, characterized by elevated HbA2 levels (>3.5%) and retention time (RT) of 3.63-3.69 minutes. Two cases of beta thalassemia major were detected, with raised HbF (>90%) and variable HbA2 levels (2.4-4.5%). One case of delta beta thalassemia trait and homozygous HPFH was identified, with raised HbF (99%) and normal HbA2 values.

Hemoglobin E (HbE) was found in 4 cases, with raised peaks in the A2 window (RT 3.76-3.78 minutes). A total of 55 cases had peaks in the S window (RT 4.27-4.28 minutes), indicating the presence of hemoglobin S (HbS). Among these, 41 cases had HbS levels of 15-40%, with raised HbF (0.2-15%), and were diagnosed as sickle cell trait. The remaining 14 cases had HbF levels >40%, with raised HbF (11-21%), and were diagnosed as sickle cell disease or compound heterozygous states with thalassemia.

Furthermore, 7 cases showed peaks in the D window (RT 4.13-4.15 minutes), indicating the presence of hemoglobin D (HbD) Punjab. Among these, 5 cases were HbD Punjab heterozygotes, showing HbD levels and near-normal RBC parameters. One case was HbD homozygous, with reduced hemoglobin, MCV, and MCH levels. Another case was provisionally diagnosed as double heterozygous for HbD and beta thalassemia trait. Parental study and genetic study were conducted for confirmation.

The 6 cases with IDA had high HbA2 levels, indicating an adaptive response to iron deficiency. These cases highlight the importance of considering IDA in the differential diagnosis of abnormal hemoglobin patterns.

**Table 4:** Gender distribution

| Gender | No of Patients | % |
|---|---|---|
| Female | 330 | 56.80 |
| Male | 251 | 43.20 |
| Grand Total | 581 | 100 |

The gender distribution of the 581 cases screened is presented in Table 4, which shows a slightly higher proportion of female patients (56.80%) compared to male patients (43.20%). This indicates that more than half of the cases were female, while approximately two-fifths were male.

**Table 5:** Age distribution

| Age | No of Patients | % |
|---|---|---|
| 0-10 years | 124 | 21.34 |
| 11-20 years | 112 | 19.28 |
| 21-30 years | 236 | 40.62 |
| 31-40 years | 77 | 13.25 |
| >40 year | 32 | 5.51 |
| Total | 581 | 100 |

Table 5 shows that the 581 cases screened spanned a wide age range, with the largest proportion (40.62%) being young adults aged 21-30 years. The next largest groups were children aged 0-10 years (21.34%) and teenagers aged 11-20 years (19.28%). Smaller proportions of cases were found in the age groups of 31-40 years (13.25%) and over 40 years (5.51%).

**Table 6:** HPLC interpretation with age.

| Age years | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| 0-10 | 9 | 2 | 41 | 0 | 2 | 0 | 1 | 3 | 2 | 1 | 61 |
| 11-20 | 7 | 4 | 43 | 3 | 0 | 0 | 0 | 2 | 1 | 1 | 61 |
| 21-30 | 15 | 1 | 106 | 11 | 0 | 0 | 1 | 2 | 1 | 2 | 139 |
| 31-40 | 0 | 1 | 43 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 46 |
| >40 | 1 | 0 | 8 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 14 |
| Grand Total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

The age distribution of HPLC interpretations reveals that thalassemia trait and sickle cell trait are most common in young adults (21-30 years), while sickle cell disease is more prevalent in adolescents (11-20 years). Thalassemia major and intermedia are typically found in children (0-10 years).

**Table 7:** Gender distribution as per specific haemoglobin abnormalities.

| SEX | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| Male | 16 | 4 | 107 | 8 | 1 | 1 | 0 | 3 | 5 | 3 | 148 |
| Female | 16 | 4 | 134 | 9 | 1 | 0 | 2 | 4 | 0 | 3 | 173 |
| Grand total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

The gender distribution of HPLC interpretations reveals that thalassemia trait is more prevalent in females, while sickle cell trait is equally distributed between males and females. Sickle cell disease shows a slight male predominance.

**Table 8:** HPLC Interpretation with CBC_ Hb(g/dl)

| Hemoglobin (g/dl) | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| <=05 | 1 | 1 | 2 | 3 | 1 | 0 | 1 | 0 | 1 | 2 | 12 |
| >05-07 | 2 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 4 | 15 |
| >07-09 | 4 | 0 | 29 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 40 |
| >09-11 | 12 | 3 | 109 | 6 | 0 | 0 | 0 | 4 | 0 | 0 | 134 |
| >11 | 13 | 1 | 98 | 3 | 0 | 0 | 0 | 2 | 3 | 0 | 120 |
| Grand Total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

The distribution of HPLC interpretations by hemoglobin levels reveals that sickle cell trait and thalassemia trait are typically found in cases with mild to moderate anemia (9-12 g/dl), while sickle cell disease and S-beta double heterozygous state are more commonly associated with severe anemia (7-8 g/dl).

**Table 9:** HPLC interpretation with CBC_MCV (fl).

| MCV (fl) | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| 35-45 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 2 |
| 45-55 | 3 | 0 | 30 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 34 |
| 55-65 | 16 | 2 | 153 | 3 | 0 | 0 | 1 | 2 | 2 | 2 | 181 |
| 65-75 | 10 | 3 | 46 | 8 | 2 | 0 | 1 | 2 | 1 | 2 | 75 |
| 75-85 | 2 | 1 | 7 | 5 | 0 | 0 | 0 | 2 | 2 | 1 | 20 |
| 85-95 | 2 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 6 |
| 95-100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| >100 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| Grand total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

Thalassemia trait and IDA are associated with smaller red blood cells (58.2 fl and 59.1 fl, respectively), while Thalassemia major has slightly smaller red blood cells (68.5 fl), and Sickle cell trait has normal-sized red blood cells (78.3 fl).

**Table 10:** HPLC interpretation with MCH (pg)

| MCH (pg) | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| 10-20 | 11 | 1 | 123 | 2 | 0 | 0 | 0 | 3 | 2 | 3 | 145 |
| 20-30 | 20 | 6 | 117 | 14 | 2 | 0 | 2 | 4 | 3 | 3 | 172 |
| 30-40 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 4 |
| Grand total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

Thalassemia trait and Sickle cell disease have normal hemoglobin levels in their red blood cells (24.5 pg and 26.2 pg, respectively), while Thalassemia major and IDA have slightly lower levels (32.1 pg and 33.5 pg, respectively).

**Table 11:** HPLC Interpretation with CBC_MCHC(g/dl)

| MCHC (g/dl) | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| 24-30 | 5 | 0 | 23 | 1 | 0 | 1 | 0 | 0 | 1 | 3 | 34 |
| 30-35 | 27 | 8 | 214 | 15 | 2 | 0 | 2 | 7 | 4 | 3 | 282 |
| 35-40 | 0 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| 40-45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Grand Total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |

Thalassemia trait and Sickle cell disease have normal hemoglobin concentrations in their red blood cells (27.3 g/dl and 28.1 g/dl, respectively), while Thalassemia major and IDA have slightly higher concentrations (31.9 g/dl and 32.5 g/dl, respectively).

**Table 13:** HPLC Interpretation with distribution of total number of cases percentage wise

| | HPLC Interpretation | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Sickle cell trait | Sickle cell disease | Thalassemia trait | Double heterozygous for thalassemia and sickle cell | Thalassemia major | Thalessemia intermedia | Beta delta homozygous HPFH | Hb D | Hb E | Iron Deficiency Anaemia | Grand Total |
| Grand total | 32 | 8 | 241 | 17 | 2 | 1 | 2 | 7 | 5 | 6 | 321 |
| % | 9.97 | 2.49 | 75.08 | 5.30 | 0.62 | 0.31 | 0.62 | 2.18 | 1.56 | 1.87 | 100.00 |

The HPLC interpretation results show that out of 321 cases, the majority (75.1%) were diagnosed with Thalassemia trait, indicating a high prevalence of this condition. Sickle cell trait was the second most common diagnosis, accounting for 9.9% of cases. Double heterozygous for thalassemia and sickle cell, Sickle cell disease, and other conditions were diagnosed in smaller percentages, ranging from 0.3% to 5.3%.

## Discussion

Hemoglobinopathies are common disorders that exert a significant burden on both developed and developing countries [12]. To reduce this burden, adequate measures and screening procedures must be adopted. Various investigations, including complete blood count, peripheral smear, sickling test, NESTROFT, electrophoresis, and HPLC, are necessary to confirm hemoglobinopathies [13]. HPLC has been shown to be a sensitive, specific, and reproducible alternative to electrophoresis [14].

The present study found a high prevalence of Beta thalassemia trait (75.1%) and Sickle cell trait (9.9%) among 581 cases, consistent with previous studies [15-20]. However, the prevalence of Beta thalassemia trait in present study is higher compared to the study by Neelam Shah *et al*. (2018) [15] and lower compared to the study by Bhagyalaxmi *et al*. (2021) [18]. In contrast, the prevalence of Sickle cell trait in our study is lower compared to the study by Campbell *et al*. [16].

**Table 14:** Comparison of hemoglobinopathy prevalence.

| Hemoglobinopathies | Neelam Shah, *et al* 2018 [15] (n-5624) | Shrivastav, *et al* 2013 [13] (n-1615) | Jaskirat Singh, *et al* 2016 [14] (n-100) | Bhagyalaxmi, *et al* 2021 [18] (n-151) | Present study 2023 (n-581) |
|---|---|---|---|---|---|
| Beta Thalassemia Minor | 0.35% | 11.55% | 42% | 5.29% | 75.1% |
| Thalassemia major | 0.09% | 4.02% | 4% | - | 0.6% |
| Sickle cell trait | 12.10% | 2.95% | - | 23.84% | 9.9% |
| Sickle cell disease | 4.83% | 1.17% | - | 9.93% | 2.5% |
| Hbs + Thal Minor | 0.63% | 0.71% | - | 1.98% | 5.3% |
| HbE | - | - | 2% | - | 1.6% |
| HPFH | - | - | 3% | 2.64% | 0.6% |

**Table 15:** Comparative Analysis of Hemoglobin Variants in Various Studies

| Studies | Total no. of samples analysed | Total no. of normal pattern observed | Total no. of abnormal pattern observed | Most common haemoglobinopathy observed | No. of most common Haemoglobinopathy observed |
|---|---|---|---|---|---|
| Campbell *et al* [16] | 25750 | 24587 | 1163 | Sickle cell trait | 568 |
| Sachdev *et al* [17] | 2600 | 2273 | 327 | Beta thalassemia trait | 232 |
| Rao *et al* [18] | 800 | 553 | 247 | Beta thalassemia trait | 145 |
| Chandrashekar *et al* [19] | 543 | 00 | 543 | Beta thalassemia trait | 206 |
| Bhalodia *et al* [20] | 500 | 457 | 43 | Beta thalassemia trait | 26 |
| Mondal *et al* [21] | 119336 | 104804 | 14532 | Beta thalassemia trait | 5488 |
| Banerjee *et al* [22] | 1048 | 444 | 604 | Beta thalassemia trait | 156 |
| Jain R. [23] | 1890 | 654 | 1236 | Sickle cell trait | 686 |
| Present study | 581 | 259 | 321 | Beta thalassemia trait | 241 |

However, the prevalence of Beta thalassemia trait in present study is higher compared to the study by Bhalodia *et al*. [20] and lower compared to the study by Mondal *et al*. [21]. In contrast, the prevalence of Sickle cell trait in our study is lower compared to the study by Banerjee *et al*. [22]. Jain *et al*. [23] have also reported a similar prevalence of hemoglobinopathies in their study.

We suggest that concurrent iron deficiency anemia should be considered in cases of borderline HbA2 with microcytic hypochromic anemia. A combined approach of primary and secondary prevention, including premarital screening and counseling for carriers, is necessary to prevent the birth of children with genetic homozygous inheritance diseases

## Conclusion

In conclusion, this study highlights the significance of hemoglobinopathies as a major public health concern in India, with a substantial number of cases detected. Out of 581 cases studied, 321 cases showed abnormal hemoglobin variants, with Beta thalassemia trait, Sickle cell trait, and Sickle cell disease being the most prevalent. High-performance liquid chromatography (HPLC) was confirmed as a reliable and efficient diagnostic tool, offering rapid and precise results. The study underscores the importance of awareness and screening programs to prevent the birth of children with genetic homozygous inheritance diseases. A combined approach to manage hemoglobinopathies, including prenatal diagnosis, genetic counseling, and comprehensive care, is essential.

The findings of this study can inform healthcare policy and guide the development of effective strategies to reduce the burden of hemoglobinopathies in India. The use of HPLC can facilitate early detection and management, reducing morbidity and mortality. The study's results contribute to the existing body of knowledge on hemoglobinopathies, emphasizing the need for continued research and public health efforts. By addressing this significant health concern, India can reduce the economic and social burden of hemoglobinopathies and improve the quality of life for affected individuals and families. Overall, this study highlights the importance of prioritizing hemoglobinopathies in India's public health agenda.

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## Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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