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**Original Research Article**

# Role of high-performance liquid chromatography in detection of hemoglobinopathies: a cross-sectional study

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## ABSTRACT

**Background:** Hemoglobinopathies are a group of inherited disorders affecting the public health in many parts of the world, particularly in South Gujarat region for structure or production of haemoglobin (Hb). Hemoglobinopathies are disorders having high mortality ratio in regions with high prevalence carrier state. Objectives were to determine the frequency of hemoglobinopathies in south Gujarat community by using high performance liquid chromatography (HPLC) test, to study haemoglobin disorder among all ages and gender wise distribution of haemoglobinopathies, to study the incidence of haemoglobinopathies in different castes in patients and to compare the results of present study with the results of other authors similar studies.
**Methods:** Total 740 cases were observed on Bio-Rad D-10 HPLC, a platform for screening haemoglobin variant and hemoglobinopathies. Cases that were diagnosed over one year at SMIMER hospital, Surat and were taken for study. Each case evaluated with clinical features, hematological parameters and its geographical pattern.
**Results:** The cases included a series of hemoglobinopathies like sickle cell disease (SCD), sickle cell trait (SCT) HbS, beta-thalassemia trait/major/minor and intermedia, HbE disease and other rare diseases etc. Common clinical condition included low Hb, fever, cold, joint pain, abdominal pain, splenomegaly. Family study was taken into consideration for accurate diagnosis.
**Conclusions:** Studying spectrum of hemoglobinopathies diagnosed by HPLC helps improving the prevalence and distribution of various haemoglobin disorders. It gives accurate quantitative measurements. Early diagnosis and management of hemoglobinopathies improve quality of life and reduce the risk of morbidity.

**Keywords:** Hemoglobinopathies, Haemoglobin, HPLC, HbS

## INTRODUCTION

### *Haemoglobin disorders*

There are two main groups of inherited disorders of Hb: Structural variants-defects in the structure of a globin chain. Thalassemia's-defects in the synthesis of a globin chain. There is some overlap between these groups, hereditary persistence of fetal hemoglobin (HPFH) is related to the thalassemias, although its phenotype is not clearly thalassaemic. Most thalassemia involves the alpha or beta globin chain.[1]

### *Normal HbS*

The heme group is identical in all Hb. The globin part of the molecule is a tetramer, made up of two dimers; each dimer contains an α-like and a β-like globin. Beyond the embryonic period, there is one type of α-like globin that forms three distinct Hb with the three different β-like globins.[1]

The patients suffering from beta-thalassemia major or Hb E, do not survive for more than 5 years without blood transfusion. Red blood cells become hard and sticky and get stuck in small blood vessels, it is characterized by formation of long chains of hemoglobin when deoxygenated within capillary beds, resulting in sickle-shaped red blood cells.[2,3] Patients having hemoglobinopathies are presented with wide range of clinical manifestation which may vary from asymptomatic states to severe, lifelong, transfusion-dependent anemia with multi organ involvement and severely reduced life expectancy.[4] HPLC provides highly accurate and reproducible measurements of analytes, which are essential for diagnosing diseases, monitoring therapeutic drug levels and assessing nutritional status.[5] Proper screening for carriers in child bearing age and in children helps in early detection of heterozygote state.[10] In many developing countries haemoglobinopathies are becoming more evident and this prominence is the driving force for the acceptance and introduction of genetic services.[11] When an abnormality of Hb function (increased or decreased oxygen affinity) or stability (unstable Hb variants) is suspected from the phenotype, special confirmatory tests (determination of p50, Heinz body prep and isopropanol or heat stability tests) can be useful or family studies are also helpful in certain cases.[12]

## METHODS

Inclusion criteria include all age group having significant family history, clinical features or anemic.

Exclusion criteria exclude babies less then 6 month as it contains fetal Hb, blood transfusion in less then 3 months or any post BT sample.

A cross sectional study of total 740 cases were observed and studied. Cases that were diagnosed over one year from January 2025 to December 2025 at Surat Municipal Institute of Medical Education and Research Hospital were taken for study. After receiving blood samples for HPLC and detailed clinical history, significant hematological parameters like Hb, white blood cell (WBC), platelets, mean corpuscular volume (MCV) were noted.

After satisfying criteria blood sample is run for HPLC test in BIO-RAD D-10 machine. Chromatography is a technique for separating mixtures into their components in order to analyse, identify, purify, and quantify the mixture or components based on differential partitioning between the mobile and stationary phases. The various constituents of the mixture travel at different speeds, causing them to separate. It requires 2 ml of blood sample- each sample takes 6 ½ minutes for analysis. The samples are injected into the analysis stream and separated by cation exchange cartridge using a phosphate ion gradient generated by mixing 2 buffers of different ionic strengths to elute the different hemoglobins. A dual wavelength filter photometer monitors the eluent from the cartridge as it passes through the photometer cell. Changes in the optical density at 415 nm are measured. A secondary filter at 690 nm corrects the effect caused by mixing buffers of different ionic strengths.

The data is processed- chromatogram produced-different peaks identified at different windows.

## RESULTS

Both indoor care patients and out patients samples were taken. The patient age ranges from 6 month to 60 years.

HPLC data range should be according to the following values shown in the Tables.[9]

Total area range was 1 million to 5 million.

Peak shape should be sharp and symmetrical, no degradation peak at end of the run.

### *Hb A2*

A normal minor component of adult Hb ranges from 1.5-11.4%.

### *Fetal haemoglobin*

Fetal Hb (HbF) is the predominant Hb present in the fetus and newborn ranges <10%.

### *Haemoglobin S and C*

The abnormal Hb present in SCDs ranges from 30-40% in trait and 70-90% in SCD condition.

Combined area of HbS and HbC ≥60% should be suspected of having a homozygous variant or variant beta thalassemia phenotype.

HPLC cases shows a normal haemoglobin pattern in 495 cases (66.89%), while 245 cases (33.11%) suggesting abnormal haemoglobin patterns. Haemoglobinopathies, such as homozygous SCD, double heterozygous states, HbE variants, HbD Punjab, α-thalassemia and HPFH, are diagnosed in smaller numbers.

Among the 105 cases of SCT, the majority of cases shows HbS levels between 31-40% (42.86%), followed by 21-30% (28.57%) and 11-20% (14.29%).

Among cases of SCT, HbA2 levels are within the range of 0-3.5% in the majority of cases (85.71%). HbA2 levels of 3.5-5% were seen in 12.38% of cases, while only 1.91% showed Hb A2 levels above 5%.

Among the 37 cases of SCD, the majority showed HbS levels between 61-70% (32.43%), followed by 71-80% (27.03%).

In patients with SCD, HbA2 levels were between 0-3.5% in 67.57% of cases. HbA2 levels of 3.5-5% were observed in 27.03% of cases, whereas 5.41% had HbA2 levels above 5%.

Among the total 37 cases of SCD, HbF levels were below 10% in 32.43% of cases. HbF levels above 30% were observed in 18.92% of cases.

The highest number of cases was observed in the 13-36-year age group, with 386 cases, followed by the 0-12-year group with 180 cases and the 37-60-year group with 174 cases. The overall distribution was nearly comparable between males and females.

Sickle cell disorders were more frequently observed among Scheduled Tribe, whereas beta-thalassemia trait was relatively more common among the Hindu General and Muslim groups.

Sickle cell disorders were more frequently observed among Scheduled Tribe, whereas beta-thalassemia trait was relatively more common among the Hindu General and Muslim groups.

**Table 1: Approach to diagnosis of haemoglobinopathies with HPLC.**

| Final diagnosis | Elution pattern of HPLC | N | Percentage (%) |
|---|---|---|---|
| **Normal HPLC pattern** | A | 495 | 66.89 |
| **SCT** | A, S | 105 | 14.19 |
| **Beta thalassemia trait** | A, A2 | 52 | 7.03 |
| **SCD** | S, F | 37 | 5.00 |
| **Homozygous sickle cell** | S | 15 | 2.03 |
| **Double heterozygous S and T** | A, S, A2 | 10 | 1.35 |
| **Sickle cell heterozygous** | A, S | 4 | 0.54 |
| **Compound heterozygous of sickle and beta Thal** | A, S, A2 | 3 | 0.41 |
| **Beta thalassemia major** | F | 2 | 0.27 |
| **Iron deficiency anemia** | A | 2 | 0.27 |
| **Compound heterozygous HbS and beta Thal** | A, S, A2 | 2 | 0.27 |
| **HbD Punjab heterozygous** | A, D | 1 | 0.14 |
| **Alpha thalassemia** | A | 1 | 0.14 |
| **HbE heterozygous** | A, E | 1 | 0.14 |
| **HbE homozygous** | E | 1 | 0.14 |
| **Compound heterozygous HbE and beta thal** | A, E, A2 | 1 | 0.14 |
| **SCD with HPFH** | S, F | 1 | 0.14 |
| **Sickle cell heterozygous + beta Thal trait** | A, S, A2 | 1 | 0.14 |
| **HPFH** | A, F | 1 | 0.14 |
| **SCD + Thal major** | S, F | 5 | 0.68 |
| **Total** | | 740 | 100.00 |

**Table 2: Percentage of Hb S in cases of SCT.**

| Age Hb S% (in years) | N | Percentage |
|---|---|---|
| **0-10** | 5 | 4.76% |
| **11-20** | 15 | 14.29% |
| **21-30** | 30 | 28.57% |
| **31-40** | 45 | 42.86% |
| **41-50** | 10 | 9.52% |
| **Total** | 105 | 100.00 |

**Table 3: Percentage of Hb A2 in cases of SCT.**

| Hb A2 % | N | Percentage |
|---|---|---|
| **0-3.5** | 90 | 85.71% |
| **3.5-5** | 13 | 12.38% |
| **>5** | 2 | 1.91% |
| **Total** | 105 | 100.00 |

**Table 4: Percentage of Hb S in cases of SCD.**

| Hb S % | N | Percentage |
|---|---|---|
| **<50** | 0 | 0.00 |
| **51-60** | 8 | 21.63% |
| **61-70** | 12 | 32.43% |
| **71-80** | 10 | 27.03% |
| **81-90** | 5 | 13.51% |
| **91-100** | 2 | 5.41% |
| **Total** | 37 | 100.00 |

**Table 5: Percentage of Hb A2 in cases of SCD.**

| Hb A2 % | N | Percentage |
|---|---|---|
| **0-3.5** | 25 | 67.57% |
| **3.5-5** | 10 | 27.03% |
| **>5** | 2 | 5.41% |
| **Total** | 37 | 100.00 |

**Table 6: Percentage of Hb F in cases of SCD.**

| Hb F % | N | Percentage |
|---|---|---|
| **0-10** | 12 | 32.43% |
| **11-20** | 10 | 27.03% |
| **21-30** | 8 | 21.62% |
| **31-40** | 5 | 13.51% |
| **>40** | 2 | 5.41% |
| **Total** | 37 | 100.00 |

**Table 7: Age and gender wise distribution of haemoglobinopathies.**

| Type of haemoglobinopathies | Age and gender wise distribution (in years) | | | | | | Total | Percentage |
|---|---|---|---|---|---|---|---|---|
| | 0-12 | | 13-36 | | 37-60 | | | |
| | M | F | M | F | M | F | | |
| **Normal HPLC** | 60 | 65 | 120 | 130 | 58 | 62 | 495 | 66.89% |
| **SCT** | 8 | 10 | 28 | 32 | 12 | 15 | 105 | 14.19% |
| **Thalassemia trait** | 5 | 6 | 13 | 15 | 6 | 7 | 52 | 7.03% |
| **SCD** | 6 | 7 | 10 | 8 | 3 | 3 | 37 | 5.00% |
| **Homozygous sickle cell** | 3 | 3 | 4 | 3 | 1 | 1 | 15 | 2.03% |
| **Double heterozygous S and thalassemia** | 1 | 1 | 3 | 2 | 1 | 2 | 10 | 1.35% |
| **Heterozygous sickle cell** | 0 | 1 | 1 | 1 | 1 | 0 | 4 | 0.54% |
| **Compound heterozygous sickle and beta thalassemia** | 0 | 0 | 1 | 1 | 1 | 0 | 3 | 0.41% |
| **Thalassemia major** | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 0.27% |
| **Iron deficiency anemia** | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0.27% |
| **Compound heterozygous HbS and beta thalassemia** | 0 | 0 | 1 | 1 | 0 | 0 | 2 | 0.27% |
| **HbD Punjab heterozygous** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **Alpha thalassemia** | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0.14% |
| **HbE heterozygous** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **HbE homozygous** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **Compound heterozygous HbE and beta thalassemia** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **SCD with HPFH** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **Sickle cell heterozygous + beta Thal trait** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **HPFH** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0.14% |
| **SCD+Thal major** | 1 | 1 | 1 | 1 | 1 | 0 | 5 | 0.68% |
| **Total** | 85 | 95 | 190 | 196 | 84 | 90 | 740 | 100% |

**Table 8: Caste wise distribution of haemoglobinopathies.**

| Type of haemoglobinopathies | Hindu general category | Schedule caste | Schedule tribe | Muslims | Total |
|---|---|---|---|---|---|
| **Normal HPLC** | 180 | 95 | 145 | 75 | 495 |
| **SCT** | 20 | 15 | 60 | 10 | 105 |
| **Beta thalassemia trait** | 21 | 5 | 5 | 21 | 52 |
| **SCD** | 5 | 4 | 25 | 3 | 37 |
| **Sickle cell homozygous** | 2 | 2 | 10 | 1 | 15 |
| **Double heterozygous S and thalassemia** | 2 | 1 | 6 | 1 | 10 |
| **Heterozygous sickle cell** | 1 | 1 | 1 | 1 | 4 |
| **Compound heterozygous sickle and beta thalassemia** | 1 | 0 | 2 | 0 | 3 |
| **Thalassemia major** | 1 | 0 | 0 | 1 | 2 |
| **Iron deficiency anemia** | 1 | 0 | 0 | 1 | 2 |
| **Compound heterozygous HbS and beta thalassemia** | 1 | 0 | 1 | 0 | 2 |
| **HbD Punjab heterozygous** | 1 | 0 | 0 | 0 | 1 |
| **Alpha thalassemia** | 0 | 0 | 0 | 1 | 1 |
| **HbE heterozygous** | 0 | 0 | 0 | 1 | 1 |
| **HbE homozygous** | 0 | 0 | 0 | 1 | 1 |
| **Compound heterozygous HbE and beta thalassemia** | 0 | 0 | 0 | 1 | 1 |
| **SCD with HPFH** | 0 | 0 | 1 | 0 | 1 |
| **Sickle cell heterozygous + beta Thal trait** | 0 | 0 | 1 | 0 | 1 |
| **HPFH** | 1 | 0 | 0 | 0 | 1 |
| **SCD+Thal major** | 1 | 1 | 2 | 1 | 5 |
| **Total** | 238 | 124 | 259 | 119 | 740 |

**Table 9: Comparison of distribution of various haemoglobinopathies of present study with other published studies.**

| Type of haemoglobinopathies | Vasaikar[6] | Balgir[7] | Jain[8] | Udin[13] | Rao[14] | Present study (%) |
|---|---|---|---|---|---|---|
| **SCT** | 82.7 | 45.3 | 2.1 | 1.1 | 4.4 | 42.86 |
| **SCD** | 9.9 | 11.6 | 0.5 | 0.0 | 1.6 | 15.10 |
| **Beta thalassemia trait** | 6.7 | 27.8 | 55.9 | 36.9 | 58.7 | 21.22 |
| **Thalassemia major** | 0.1 | 8.1 | 9.5 | 0.9 | 9.3 | 0.82 |
| **Double heterozygous for HbS and beta thalassemia** | 0.4 | 2.5 | 1.3 | 0.0 | 2.4 | 4.08 |
| **HbD trait** | 0.1 | 0.3 | 0.3 | 0.0 | 2.8 | 0.41 |
| **Double heterozygous for HbS and HbD** | 0.0 | 0.3 | 0.1 | 0.0 | 0.4 | 0.00 |
| **HbE homozygous** | 0.0 | 0.4 | 0.6 | 15.8 | 0.4 | 0.41 |
| **Suspected α-thalassemia** | 0.0 | 0.0 | 0.0 | 0.0 | 4.4 | 0.41 |
| **HPFH/delta beta thalassemia trait** | 0.0 | 1.3 | 0.7 | 0.9 | 2.4 | 0.41 |
| **Hereditary persistent fetal Hb heterozygous** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 |
| **Beta thalassemia intermedia** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.00 |
| **Other haemoglobinopathies** | 0.1 | 2.4 | 28.9 | 44.4 | 13.0 | 14.29 |

* Normal cases are excluded for comparison purpose and percentage of positive cases are taken accordingly in all other studies.

## DISCUSSION

The present cross sectional study for 740 cases evaluated by HPLC over a one-year period and gives a wide spectrum of hemoglobinopathies in South Gujarat. The patient flow is mainly from south Gujarat where some community has inherited sickle cell and thalassemia hemoglobinopathy, as it common in south Gujarat community. So, there is a high frequency of sickle cell anaemia cases in present study. In India, the frequency of beta thalassemia ranges from 3.5% to 15% in general population.[8] Among all cases, 245 (33.11%) showed abnormal haemoglobin patterns. Among this, sickle cell disorders constituted the largest group of

hemoglobinopathies in the present study. SCT was constitute 14.19% of cases, while SCD constituted for 5.0%. Conclusively, sickle cell-related disorders represented more than one-fifth of all abnormal cases. The percentage of carriers of thalassaemia is greater than that of carriers of sickle-cell anaemia, but because of the higher frequency of the sickle-cell gene in certain regions, the number of affected births is higher than with thalassaemia.[11] This high prevalence is consistent with the known epidemiological distribution of sickle haemoglobin in tribal populations of South Gujarat, where the sickle gene is highly prevalent. Beta-thalassemia trait was the most common hemoglobinopathy, accounting for 21.22% of cases. Age distribution revealed that most hemoglobinopathy cases were diagnosed in the 13-36-year age group with females (26.4%) and males (25.6%) affecting in each hemoglobinopathy. The caste-wise distribution showed a higher frequency of sickle cell disorders among scheduled tribe populations, whereas beta-thalassemia trait was relatively common among Hindu General and Muslim communities. Among patients with SCD, HbS levels were predominantly between 61% and 80%, while most patients had HbF levels below 20%. Similar findings were reported by Vasaikar and Jain although the frequencies varied between studies.[6,8] The frequencies of SCT (42.86%) were lower than those reported in the comparative studies. but SCD (15.10%) were higher than those reported in the comparative studies. While Roa has highest beta thalassemia trait(58.7%) in its study comparative to others.[13] The prevalence of beta thalassemia trait (21.22%) in the present study was lower than that reported in most of the comparative studies, particularly those by Rao et al (58.7%), Jain et al (55.9%), and Udin et al (36.9%).[8,13,14] Other haemoglobinopathies constituted 14.29% of cases in the present study, showing the wide spectrum of haemoglobin variants detected by HPLC. Rare disorders such as HbD Punjab, HbE variants, α-thalassemia, HPFH, and compound heterozygous conditions are also present. Most common limitation for this study is that, it was a single-center study with a limited duration and lacked molecular confirmation of hemoglobin variants. So furthermore, clinical follow-up and family screening required.

## CONCLUSION

The findings confirms the importance of HPLC as a reliable screening and diagnostic tool for the detection and characterization of hemoglobinopathies. HPLC provides excellent separation of complex mixtures, even for compounds with very similar chemical properties that are difficult to separate using other techniques. It give accurate quantitative measurements and early diagnosis and management of hemoglobinopathies to improve quality of life and reduce the risk of morbidity. HbA2 values were useful in identifying β-thalassemia carriers and compound heterozygous conditions, highlighting the advantage of HPLC over other electrophoretic methods. The detection of rare conditions such as HbE heterozygous and homozygous states, HbD Punjab (Varient of hemoglobinopathy), HPFH, and compound heterozygous disorders further demonstrates the diagnostic capability of HPLC in differentiating a wide range of hemoglobin variants. Advice should be given for further family study for accuracy of diagnosis. The identification of thalassemia carriers is important because carrier detection and genetic counselling can significantly reduce the incidence of severe thalassemia disorder through prenatal and premarital screening programs, antenatal testing, and investigation of anemia during early adulthood.

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