Original Article

Prevalence of Hemoglobinopathies in Different Age Groups- A One Year Study in Tertiary Care Centre

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Abstract

Aim and background: Hemoglobinopathies such as thalassemia and sickle cell disease are among the most common genetic disorders in India, with carrier rates ranging from 3% to 17%. Each year, 9,000–10,000 new β-thalassemia cases are added, and prevalence is particularly high among certain ethnic groups, scheduled tribes, and in eastern/northeastern regions with HbE. Despite 10,000–12,000 thalassemia births annually, most affected children lack adequate care. Strengthening public awareness, carrier screening, prenatal diagnosis, and access to treatment is essential, with HPLC serving as preferred diagnostic tool.

Material and method: This one-year observational study at PGIMS Rohtak included all samples submitted for HPLC with suspected hemoglobinopathies. Each sample underwent complete hemogram and HPLC analysis to detect and characterize hemoglobin variants. Chromatogram findings were interpreted with CBC data to ensure diagnostic accuracy.

Result: Of all samples analyzed, 74.9% showed normal HPLC patterns, while 25.1% revealed abnormalities. β-thalassemia trait was the most common hemoglobinopathy (55.7%), followed by HbD Punjab heterozygous (25.8%) and HbE heterozygous (4.9%); fewer cases of β-thalassemia major, intermedia, HbE homozygous, double heterozygous HbE/β-thalassemia, and sickle cell trait were identified. Most hemoglobinopathies occurred in individuals $\leq$30 years, with a female predominance in several subtypes. Hematological profiles showed microcytic hypochromic anemia in thalassemia major, moderate indices in intermedia/trait, higher red cell parameters in HbE homozygous, and elevated HbF in thalassemia major.

Conclusion: HPLC is a rapid and reliable method for detecting hemoglobinopathies, demonstrating regional variation and distinct subtype profiles. Complementary molecular methods and genetic counselling are essential for better management and prevention.

Keywords: high-performance liquid chromatography (hplc); complete blood count (cbc); thalassemia; sickle cell disease; prevalence

Introduction

Hemoglobin is an essential protein in red blood cells that carries oxygen from the lungs to the rest of the body and helps remove carbon dioxide[1]. When the structure of hemoglobin is altered due to genetic mutations, it can lead to disorders known as hemoglobinopathies. These include conditions like thalassemia and sickle cell disease—both inherited in an autosomal recessive pattern—and are among the most common genetic disorders in India. India carries a heavy burden of

these conditions, with 3% to 17% of the population carrying abnormal hemoglobin genes. Each year, about 9,000 to 10,000 new β-thalassemia cases are added, and over 2.5 lakh people live with thalassemia or sickle cell disease, many without adequate care[2]. Some communities are more severely affected—carrier rates reaching 17% in specific ethnic groups[3]. Sickle cell disease is particularly common among scheduled tribes and backward castes, with carrier rates between 5% and 35%. Meanwhile, hemoglobin E (HbE) is frequently seen in eastern and northeastern India, where the carrier rate can be as high as 50%[4].

Estimates suggest that for every 1,000 live births in India, there about 1.2 babies are born with serious hemoglobin disorders[5]. Unfortunately, of the 10,000 to 12,000 thalassemic children born annually, only a small number receive proper treatment—mostly in cities—despite national policies like the 12th Five Year Plan including thalassemia and sickle cell care[6].

Managing these conditions effectively calls for strong public awareness, education of healthcare providers, routine screening, access to prenatal diagnosis, more day care centres, and affordable stem cell transplant facilities[7].

For diagnosis, high performance liquid chromatography (HPLC) is widely used because it offers a sensitive and accurate way to detect hemoglobin disorders. It is more efficient than traditional electrophoresis methods. Newer techniques using weak cation exchange materials have improved both speed and resolution, bringing the analysis time down to just 30 minutes[8].

Primary objective

Study prevalence of hemoglobinopathies in different age group.

Material and method

This was a descriptive observational study with a cross-sectional design, conducted from January to December 2024 in the Department of Pathology, PGIMS Rohtak. All samples from patients of all age groups suspected of hemoglobinopathies and submitted to the Clinical Pathology Department from various clinical departments were included in the study. Each sample underwent a complete hemogram, with all hematological parameters carefully evaluated. Anemic cases were further evaluated using high-performance liquid chromatography (HPLC) on the Bio-Rad Variant II system to detect the presence of hemoglobinopathies. HPLC chromatograms were interpreted according to the manufacturer's guidelines, assessing parameters such as baseline pattern, total peak area, peak profile and shape, and the relative percentages of different hemoglobin fractions. Complete blood count (CBC) results from the 5-part cell counter were reviewed alongside the chromatograms to support accurate identification of hemoglobin variants. β-thalassemia syndromes are traditionally classified into three major categories based on genetic pattern and laboratory features. The homozygous forms—β-thalassemia major and intermedia—typically show markedly elevated HbF levels (up to 90%), reduced HbA, and normal or increased HbA_2. Although β-thalassemia intermedia displays a similar hemoglobin profile, these patients are usually not dependent on regular transfusions. In contrast, β-thalassemia minor or trait (heterozygous form) is characterized by a higher RBC count ($>$4.5 million/μL), raised HbA_2 levels (4–9%), and normal HbF ($<$2%).

The HPLC pattern in HbE hemoglobinopathy varies with zygosity. HbE homozygous individuals generally show HbF levels of 2–10% and HbA_2 greater than 60%, whereas HbE heterozygotes typically show HbF $<$1% with HbA_2 values between 25–35%.

Sickle cell (HbS) disorders also present as homozygous or heterozygous states. Homozygous HbS usually demonstrates HbF $>$5%, HbA_2 $<$5%, and HbS levels exceeding 50%. In sickle cell trait (heterozygous), HbF remains $<$1%, HbA_2 $<$4%, and HbS typically ranges from 30–40%.

Among the HbD variants, HbD Punjab heterozygotes show a characteristic D-window peak (30–40%) with normal HbF and HbA_2, whereas HbD Iran heterozygotes display higher HbA_2 values (40–48%). Hb Lepore trait is suggested by HbF $<$10% and HbA_2 between 10–18%. Hereditary persistence of fetal hemoglobin (HPFH) in the heterozygous form is identified by markedly elevated HbF (5–30%) with normal HbA_2.

Normal control patterns were derived from the same dataset and included samples that demonstrated a normal HPLC profile with no detectable hemoglobinopathy."

All these defined hemoglobin patterns were used to interpret the HPLC chromatograms and classify the hemoglobinopathies detected in the present study.

Ethical considerations

Institutional Ethical Committee approval was obtained prior to the commencement of the study.

Statistical analysis

Data was entered in Microsoft excel mastersheet and analysed using SPSSv20 software. Descriptive statistics (mean, standard deviation, range, percentages) was applied wherever appropriate. Statistical analysis performed using chi square test, Pearson correlation test, independent t test, ANOVA test and ROC curve.

Result

Table 1: Distribution of HPLC patterns overall and by gender (n = 800).

HPLC Pattern	Total (n, %)	Male (n, %)	Female (n, %)
Normal	599 (74.9%)	237 (74.8%)	362 (74.9%)
Abnormal	201 (25.1%)	80 (25.2%)	121 (25.1%)
Total	800 (100%)	317 (100%)	483 (100%)

Majority of cases (74.9%) had normal HPLC reports, while abnormal patterns were observed in 25.1% of cases. The proportion of normal cases was nearly identical across genders, with 74.8% in males and 74.9% in females. Abnormal cases were slightly higher in males (25.2%) compared to females (25.1%), indicating a consistent distribution of normal and abnormal HPLC patterns across both gender.

Table 2: Spectrum of abnormal HPLC report (n=201).

HPLC Spectrum	Total No. of Case	%
β Thalassemia. Intermedia	9	4.4
β Thalassemia. Major	7	3.5
β Thalassemia. Trait	112	55.7
Double Heterozygous HbE and β Thalassemia.	2	0.99
HbD Punjab Heterozygous	52	25.8
HbE Heterozygous	10	4.9
HbE Homozygous	7	3.5
Sickle Cell Heterozygous	2	0.99

Out of 201 cases with abnormal HPLC reports, β-thalassemia trait was the most common hemoglobinopathy, observed in 112 cases (56%), followed by HbD Punjab heterozygous in 52 cases (25.8%) and HbE heterozygous in 10 cases (4.9%). β-thalassemia intermedia and β-thalassemia major were identified in 9 (4.4%) and 7 cases (3.5%), respectively, while HbE homozygous was also seen in 7 cases (3.5%).

Table 3: Spectrum of Abnormal HPLC Reports by Age and Gender (N=800).

HPLC Type	<20 years	21–40 years	>40 years	Male (n, %)	Female (n, %)
β Thalassemia Intermedia	5	4	1	4 (44.4%)	5 (55.6%)
β Thalassemia Major	5	2	0	4 (57.1%)	3 (42.9%)
β Thalassemia Trait	69	40	11	46 (41.4%)	66 (58.6%)
Double Heterozygous HbE & β Thalassemia	2	0	0	1 (50.0%)	1 (50.0%)
HbD Punjab Heterozygous	34	17	1	18 (34.6%)	34 (65.4%)
HbE Heterozygous	5	5	0	4 (40.0%)	6 (60.0%)
HbE Homozygous	4	3	0	2 (28.6%)	5 (71.4%)
Sickle Cell Heterozygous	1	1	0	1 (50.0%)	1 (50.0%)

β Thalassemia trait was the most common abnormal HPLC finding, mainly in individuals under 40, with a female predominance. Other hemoglobinopathies—including β Thalassemia intermedia/major, HbD Punjab, HbE variants, and sickle cell trait—were less frequent and showed variable age distribution, generally with higher female representation.

β Thalassemia major and intermedia cases had low hemoglobin and MCV with high HbF, while β Thalassemia trait showed mild anemia with elevated HbA_2. HbE and HbD Punjab variants exhibited variable hemoglobin levels with characteristic changes in HbF and HbA_2.

Discussion

The hemoglobinopathies are characterized by the production of structurally defective hemoglobin due to abnormalities in the formation of the globin moiety of the molecule. The inherited disorders of blood including hemoglobinopathies pose a

Table 4: Key hematological and HPLC parameters in hemoglobinopathies (Mean ± SD).

HPLC Type	No. of Cases	Hb (g/dl)	MCV (fl)	HbF (%)	HbA_2 (%)
β Thalassemia Major	7	5.51 ± 1.38	55.31 ± 3.03	92.73 ± 2.64	2.54 ± 0.83
β Thalassemia Intermedia	9	8.24 ± 2.11	57.37 ± 3.98	81.76 ± 30.07	2.18 ± 1.13
β Thalassemia Trait	112	8.65 ± 2.82	57.24 ± 5.47	1.69 ± 4.10	5.15 ± 0.92
Double Heterozygous HbE & β Thalassemia	2	4.10 ± 0.42	57.10 ± 0.85	16.25 ± 0.21	71.35 ± 0.07
HbE Homozygous	7	8.89 ± 2.70	69.10 ± 10.96	4.21 ± 8.09	49.44 ± 30.43
HbE Heterozygous	10	7.77 ± 2.56	61.47 ± 10.38	0.60 ± 0.19	27.91 ± 1.43
HbD Punjab Heterozygous	52	8.38 ± 2.92	66.39 ± 11.58	0.88 ± 0.62	2.19 ± 0.46

massive public health problem in many countries including India[2]. In India, sickle cell disease and β thalassemia represent a substantial health burden. According to the 2011 Census of India, the average prevalence of carriers of β thalassemia is 3–4%, which translates to 35 to 45 million carriers in our multi-ethnic, culturally and linguistically diverse population of 1.21 billion people, which also includes about 8% of tribal groups. The prevalence is significantly higher among a few ethnic groups (4–17%)[3]. High performance liquid Chromatography (HPLC) is a sensitive and precise method for detecting hemoglobin abnormalities. It is widely used for routine diagnosis due to its rapidity and reproducibility. If confirmation is required, it can be achieved by low-pressure macro-column chromatography, a labour-intensive procedure using low-pressure material such as CM-cellulose or an anion exchange material such as DEADE-cellulose which takes 2 to 3 days for a complete separation of hemoglobin variants. Both anion and cation exchange packing materials have been prepared for HPLC application. Anion exchange chromatography of hemoglobins using HPLC has been able to resolve some of the major hemoglobins, with improved separation over electrophoresis of HbA and HbF. The time to elute all hemoglobin fractions is relatively long at about 90 minutes. Weak cation exchange packing material provides a significant improvement in resolution and sensitivity and reduces the chromatographic time to 30 minutes[8]. Several methods are applied for the assessment of hemoglobinopathies among them, cation exchange HPLC offers the most reliable tool for early and accurate detection of various hemoglobinopathies. We found that a total of 201 patients had various hemoglobinopathies; β-thalassemia trait formed the largest subgroup of abnormal hemoglobin including 55.7%. The result of present study were compared with those of other published studies (Table 7).

Table 5: Comparative study of cases according to various hemoglobinopathies (spectrum of abnormal HPLC report N 201).

Hemoglobinopathy	Present Study (%)	Mondal et al[9] (%)	Kavitha et al[10] (%)	Zia et al[11] (%)
β-Thalassemia Intermedia	4.47	—	—	—
β-Thalassemia Major	3.5	13.6*	0.17	23.3
β-Thalassemia Trait	55.7	37.7	69	47.13
Double Heterozygous HbE/β-Thalassemia	0.99	9.5	—	—
HbD Punjab Heterozygous	25.8	0.73	4.3	5.3
HbE Heterozygous	4.9	24.8	4.1	2.2
HbE Homozygous	3.5	2.79	1.6	—
Sickle Cell Heterozygous	0.99	3.11	4.5	0.88

β-thalassemia spectrum prevalence: In the present study, β-thalassemia intermedia was observed in 4.47% of cases. β-thalassemia Major was found in 3.5% of cases, which is significantly lower than Mondal et al[9] and Zia et al[11](23.3%), but higher than Kavitha et al[10] (0.17%). The variation may be attributed to regional differences in genetic prevalence and screening methodologies. β-thalassemia trait had the highest occurrence (55.7%) in our study, which is considerably higher than Mondal et al[9] (37.7%), and Zia et al[11](47.13%) but lower than Kavitha et al[10](69%). This variation suggests that β-Thalassemia Trait is prevalent in our study population. Our study and the study by Kavitha et al[10] included cases of antenatal screening and trait. This highlights the need for genetic counselling so that hemoglobinopathies can be avoided in future progeny. In the present study, double heterozygous HbE and β-thalassemia cases were observed in 0.99%, markedly lower than the 9.5% reported by Mondal et al[9], a difference that may be attributed to the larger study cohort included in their analysis. The present study showed a high frequency of HbD Punjab heterozygous cases (25.8%), which is higher than other studies, including Mondal et al[9] (0.73%), Kavitha et al[10] (4.3%), and Zia et al[11] (5.3%). This significant difference indicates a possible geographic clustering or specific ethnic predisposition in the studied population. HbE variants: HbE heterozygous was identified in 4.9% of cases, lower than Mondal et al[9] (24.8%) but close to Kavitha et al[10] (4.1%) and Zia et al[11] (2.2%). HbE homozygous cases accounted for 3.5% in the present study, aligning with Mondal et al[9] (2.79%) but higher than Kavitha et al[10] (1.6%). Sickle cell disorders: Sickle cell heterozygous cases were found in 0.99%, which is lower than Mondal et al[9] (3.11%) and Kavitha et al[10] (4.5%), but comparable to Zia et al[11] (0.88%). The observed variation might be due to differences in demographic and genetic backgrounds of the studied populations.

In the present study, normal individuals showed a mean HbF of 1.38 ± 3.09% and HbA_2 of 2.82 ± 2.09%, consistent

with values reported by Mondal et al[9] and Phalak et al[12]. β-thalassemia intermedia cases demonstrated elevated HbF (81.7 $\pm$ 30.07%) with HbA_2 of 2.18 $\pm$ 1.13%, while β-thalassemia major cases showed markedly high HbF (92.73 $\pm$ 2.64%) and low HbA_2 (2.54 $\pm$ 0.83%). These findings contrast with Mondal et al[9] and Phalak et al[12], possibly due to transfusion effects or mutation heterogeneity. β-thalassemia trait cases exhibited low HbF (1.69 $\pm$ 4.10%) and elevated HbA_2 (5.23 $\pm$ 0.83%), in agreement with previous studies. Double heterozygous HbE/β-thalassemia showed moderately raised HbF (16.25 $\pm$ 0.21%) and unusually high HbA_2 (71.35 $\pm$ 0.07%), likely reflecting co-elution or genetic variation. HbD Punjab heterozygotes had near-normal HbF (0.88 $\pm$ 0.62%) with slightly raised HbA_2 (2.19 $\pm$ 0.46%). HbE heterozygotes demonstrated minimal HbF and elevated HbA_2 (27.91 $\pm$ 1.43%), while HbE homozygotes showed mildly raised HbF and HbA_2, consistent with known patterns. Sickle cell heterozygotes displayed HbF of 2.25 $\pm$ 0.64% and HbA_2 of 2.5 $\pm$ 0.42%, comparable to earlier reports.

HPLC is a highly rapid, sensitive, specific, reproducible and accurate technique for identifying and quantifying various hemoglobin fractions. Screening should be done in all suspected patients to find out exact prevalence of hemoglobinopathies. Possible treatment should be started before end organ damage. Genetic counselling of patient and family for next pregnancy should be done to avoid prevelance of hemoglobinopathies in their family. However, it has certain limitations. Notably, HPLC cannot detect alpha thalassemia or β thalassemia with normal HbA_2 levels. Additionally, hemoglobin variants that elute at the same retention time cannot be distinguished solely by HPLC. Therefore, molecular techniques such as PCR, amplification refractory mutation system (ARMS), and other advanced tests are necessary to identify the specific mutations responsible for abnormalities detected by HPLC.

Conclusion

In our study, β-thalassemia trait emerged as the most common hemoglobinopathy, along with notable cases of β-thalassemia major, intermedia, and HbD Punjab heterozygotes, highlighting the influence of regional and ethnic factors. High-performance liquid chromatography (HPLC) proved to be a quick and reliable tool for detecting and measuring abnormal hemoglobin types. Early screening and genetic counseling can help prevent the disease in future generations and ensure timely care for affected individuals. For certain cases that HPLC cannot fully resolve, molecular testing remains essential to pinpoint the exact genetic changes.

Abbreviations: High-performance liquid chromatography (HPLC); Complete blood count (CBC)

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