



Original Article

HPLC in the characterization of hemoglobin profile in thalassemia syndrome in a tertiary care hospital

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ABSTRACT

Introduction: Hemoglobinopathies, particularly thalassemia syndromes, are among the most common inherited disorders worldwide and pose a major health burden in India. Early and accurate differentiation between thalassemia major and minor is essential for effective screening and management. This study aimed to evaluate the prevalence of hemoglobin variants and correlate red blood cell (RBC) indices with high performance liquid chromatography (HPLC) findings.

Material and Methods: A total of 100 cases were analyzed using HPLC as the primary diagnostic modality. Demographic details and hematological parameters, including RBC indices, were assessed and correlated with hemoglobin variants.

Results: Beta thalassemia minor was the most prevalent variant (87%), followed by sickle-beta thalassemia heterozygous (6%) and other rare hemoglobinopathies. The male-to-female ratio was 1.27:1. Beta thalassemia Major was observed exclusively in the 0–10 year age group, while beta thalassemia minor was most common in the 21–30 year age group. Distinct patterns in hemoglobin levels and Mean corpuscular volume values aided differentiation among variants. The findings were consistent with previous studies.

Conclusion: HPLC combined with hematological parameters is a rapid, accurate, and reliable method for detecting and characterizing hemoglobinopathies, including compound heterozygous conditions, and is suitable for frontline screening.

Keywords: Hemoglobinopathies, HPLC, Red cell indices, Thalassemia.

INTRODUCTION

Abnormalities of hemoglobin (Hb) synthesis are among the most common inherited disorders of man and can be quantitative (thalassemia syndromes) or qualitative (Hb variants). Of these, thalassemia syndromes, particularly beta thalassemia major and certain alpha thalassemia, are serious and a major cause of morbidity.¹

The figures of the World Health Organization (WHO) estimate that ~5% of the world's population are carriers of the genetic hemoglobin disorders. Every year, there are over 42 million carriers, and more than 12,000 infants are born with major and clinically significant hemoglobinopathies. In India, the cumulative gene frequency of hemoglobinopathy is around 4.2%.¹

Globally, the percentage of carriers of thalassemia is greater than that of carriers of sickle cell

anemia (SCA), but because of the high frequency of the sickle cell gene in certain regions, the number of affected individuals at birth is higher than with thalassemia. In India, the prevalence of β -thalassemia trait and sickle cell hemoglobinopathy varies between 4-17% and 5-35%, respectively.² Worldwide migration of the human population, relatively higher frequency of consanguineous marriages in many countries, has equally contributed to the increased burden of Hb variants.

The thalassemias are a group of congenital anemias that have in common deficient synthesis of one or more of the globin subunits of the normal human hemoglobins. The primary defect is usually quantitative, consisting of the reduced or absent synthesis of normal globin chains, but there are mutations resulting in structural variants produced at a reduced rate (e.g., HbE, Hb Lepore) and mutations producing hyperunstable hemoglobin variants with a 2-thalassemia phenotype (thalassemic hemoglobinopathies). According to the chain whose synthesis is impaired, the most common thalassemias are called α , β , γ , or $\delta\beta$ -thalassemias. The identity of an Hb variant is generally inferred from its electrophoretic mobility, its quantity, and the patient's ethnic background. Family studies can be of considerable importance in elucidating the nature of disorders of Hb synthesis, but definite identification can be achieved only by DNA analysis or amino acid sequencing.

High-performance liquid chromatography (HPLC) is an excellent and powerful diagnostic tool to detect hemoglobin variants with a high degree of precision in the quantification of normal and abnormal hemoglobin fractions. Cation exchange high-performance liquid chromatography (CE-HPLC) is useful for rapid diagnosis of a varied spectrum of hemoglobinopathies. Retention time and percentage of variant hemoglobin can provide important clues in differentiating variant hemoglobin eluting in the same window. The advantage of the HPLC system is the excellent resolution, reproducibility, and quantification of several normal and abnormal hemoglobin, resulting in accurate diagnosis of various hemoglobin disorders.

The laboratory diagnosis of the Hb variants and Hemoglobinopathies is of growing importance, particularly because of the increasing requirement for antenatal diagnosis of significant disorders of globin chain synthesis.

AIMS AND OBJECTIVES

1. To differentiate between thalassemia major and minor.
2. To determine the age group incidence associated with thalassemia syndrome.
3. To determine the sex group incidence associated with thalassemia syndrome.
4. To correlate RBC indices with thalassemia syndrome.

5. To correlate the results of this study with the previous study.

Inclusion criteria

Cases will be diagnosed as thalassemia by using an HPLC analyzer in Civil Hospital, Ahmedabad.

Exclusion criteria

Exclusion criteria for the sample: rejected samples, clotted samples, and inadequate samples.

MATERIAL AND METHODS

This is a prospective study carried out in the Civil Hospital, Ahmedabad. A 2 mL EDTA blood sample. The sample is run in the HPLC-Bio-Rad variant 2 System, and the results are observed.

The present study was conducted for the detection of Hb variants by HPLC and correlated with complete blood count (CBC) and peripheral blood smear examination findings.

RESULTS

In the present study, 100 cases were studied, taking into account the exclusion criteria, and HPLC was carried out for the detection of Hb variants. The study was carried out between May 2023 and July 2023

The results of the study were as follows:

Out of 100 cases, Chart 1 shows the maximum cases of thalassemia are seen in males, with a male: female ratio of 1.27:1 (56% males and 44% females).

Chart 2 indicates that the most prevalent condition is beta thalassemia minor, which constitutes 87% of cases. Other less common conditions include: sickle-beta thalassemia heterozygous (6 cases), delta beta thalassemia trait (1 case), beta thalassemia major (2 cases), beta thalassemia intermedia (2 cases), and sickle trait with co-existence of alpha thalassemia (2 cases).

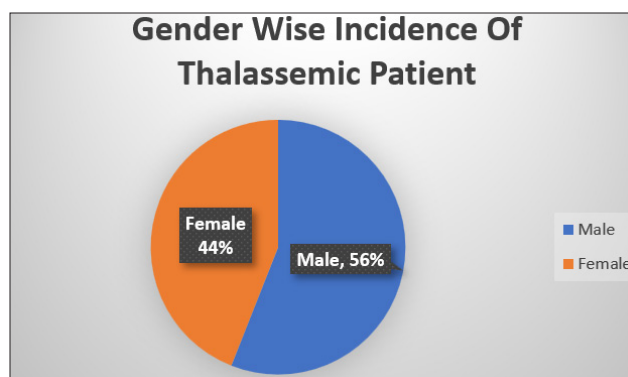


Chart 1: Gender wise distribution of thalassemic patients

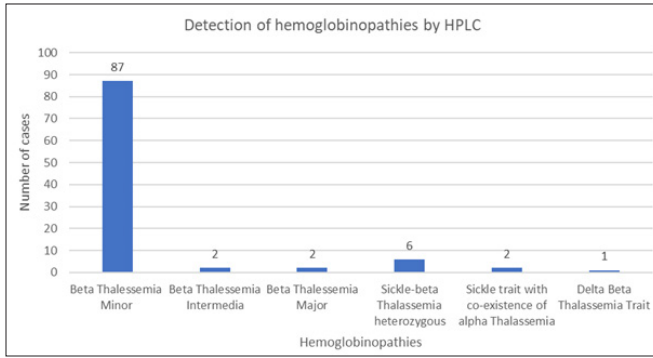


Chart 2: Detection of hemoglobinopathies by high-performance liquid chromatography (HPLC)

Table 1: Age-wise distribution of thalassemic patients

Age (years)	Beta thalassemia			Sickle-beta thalassemia heterozygous	Sickle trait with co-existence of alpha	Delta beta thalassemia trait
	Major	Minor	Inter-media			
0-10	2	7	0	0	0	1
11-20	0	28	0	4	1	0
21-30	0	35	0	1	0	0
31-40	0	14	1	0	1	0
41-50	0	1	0	1	0	0
>51	0	2	1	0	0	0

Table 1 reflects beta thalassemia major: found only in the 0-10 years age group (2 cases). Beta thalassemia minor: Peaks in the 21-30 age group (35 cases) and is spread across other age brackets. Beta thalassemia intermedia: limited to older age groups (31-40 and >51 years). Sickle-beta thalassemia heterozygous: primarily observed in the 11-20 and 31-40-year brackets. Sickle trait with co-existence of alpha thalassemia: found in the 0-10 and 11-20 age groups. Delta beta thalassemia trait: rare, with one case recorded in the 11-20 age group.

Table 2: Comparison of red blood cell indices in various hemoglobin disorders (mean, SD)

Hemo-globinopathy	HB		MCV		MCH		MCHC		HCT		RDW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beta thalassemia minor	10.33	1.65	61.7	8.45	21.85	4.23	32.71	1.5	32.51	4.91	19.65	3.19
Beta thalassemia intermedia	8.7	1.68	83.55	9.22	29.05	3.6	30.9	1.51	26.75	5.24	24.35	3.43
Beta thalassemia major	6.9	1.86	62	10	25.65	3.45	27.95	1.6	31.4	5.05	25.05	3.67
Sickle-beta thalassemia heterozygous	8.83	1.65	69.28	8.58	27.41	4.24	32.5	1.47	29.33	4.94	19.33	3.2
Sickle trait with co-existence of alpha thalassemia	9.3	1.65	76.3	8.74	24.8	4.26	32.4	1.49	28.85	4.91	26.65	3.45

Figure 1 shows representative high-performance liquid chromatography (HPLC) chromatograms for hemoglobin analysis. The images show results from a Bio-Rad CDM System for two different patient samples analyzed for hemoglobin variants. (A) HPLC profile for sample showing peak distributions including an S-window at 34.0%. (B) HPLC profile for sample 1386 highlighting elevated concentrations of Hemoglobin F (2.8%) and A2 (5.6%).

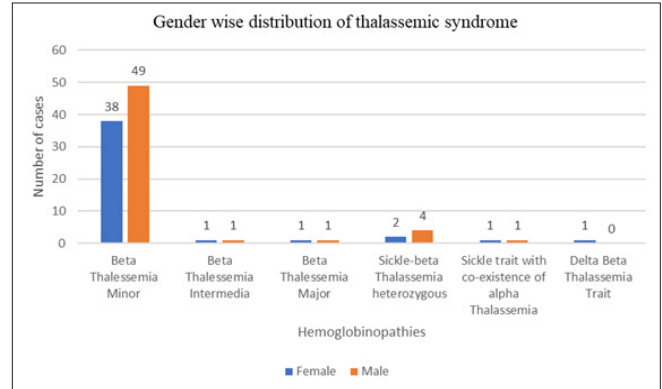


Chart 3: Gender wise distribution of thalassemic syndrome Chart 3 indicates Gender-wise distribution of thalassemia syndrome among the cases studied shows Males accounted for 56% of the cases, while females represented 44%. Indicates a slightly higher prevalence of thalassemia syndromes in males compared to females, with a male-to-female ratio of ~ 1.27:1.

Red Blood Cell indices in various hemoglobin disorders [Table 2]

Beta thalassemia minor: mild hemoglobin deficiency (mean 10.33) with low MCV (mean 61.7)

Beta thalassemia intermedia: moderate hemoglobin deficiency (mean 8.7) with high MCV (mean 83.55)

Beta thalassemia major: severe hemoglobin deficiency (mean 6.9) and low MCV (mean 62)

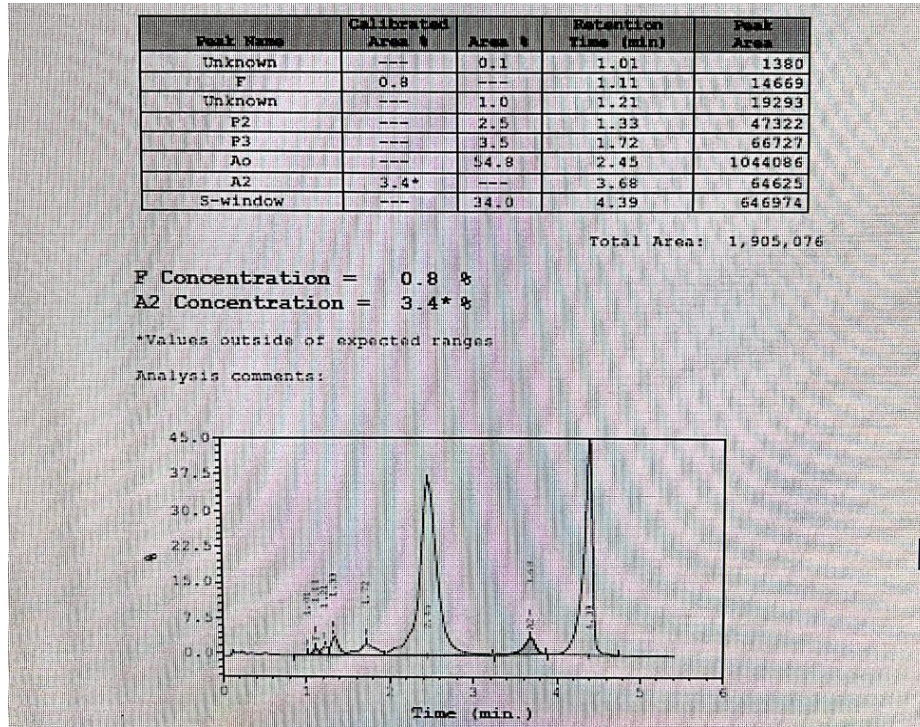


Figure 1. A) Representative high-performance liquid chromatography (HPLC) chromatogram showing peak distributions including an S-window at 34.0%.

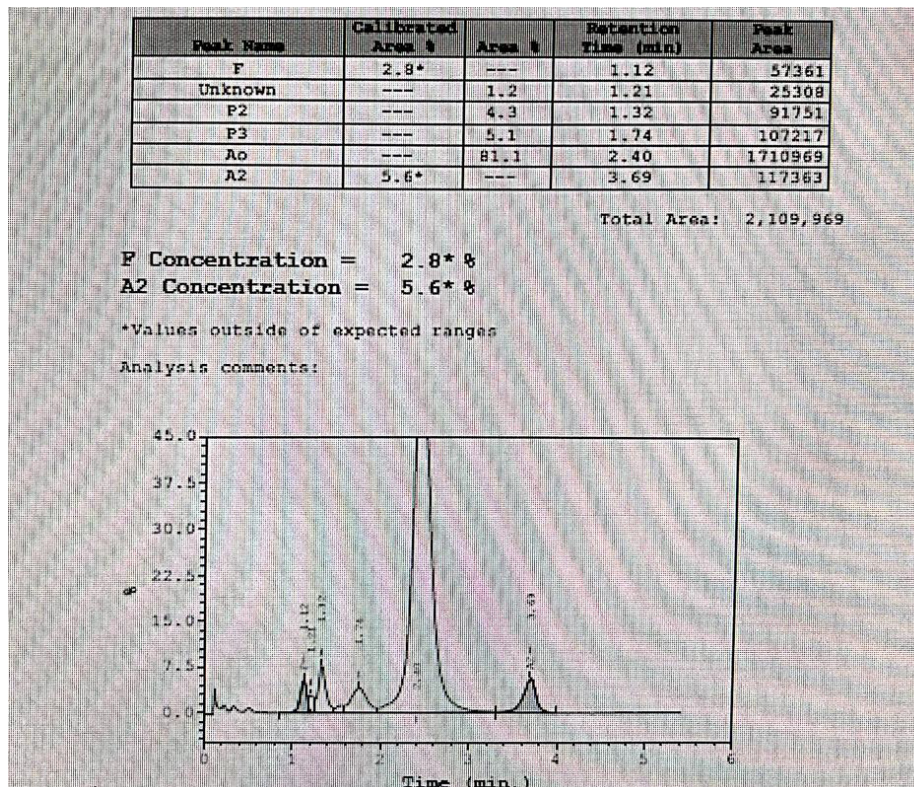


Figure 1. B) Representative high-performance liquid chromatography (HPLC) chromatogram showing elevated concentrations of Hemoglobin F (2.8%) and A2 (5.6%).

Sickle-beta thalassemia heterozygous: mild hemoglobin deficiency (mean 8.83) and low MCV (mean 69.28)

Sickle trait with co-existence of alpha thalassemia: low hemoglobin level (mean 9.3) and a moderately high MCV (mean 76.3).

DISCUSSION

Beta thalassemia major, the present study findings are 2%, which is comparable with Khera *et al.*,³ (7.23%), and significantly lower than that of Abbasi *et al.*,⁴ (41.82%), and Shrivastav *et al.*,⁵ (25.17%). Beta thalassemia minor, the present study findings are 87%, which is comparable to Khera *et al.*,³ (74.70%), and Shrivastav *et al.*,⁵ (72.32%), and comparatively higher than that of Abbasi *et al.*,⁴ (54.38%). The findings for beta thalassemia intermedia (2%) are consistent with Khera *et al.*,³ (6.02%) and Shrivastav *et al.*,⁵ (1.4%), indicating the rarity of this condition. The findings for beta thalassemia heterozygous (9%) align with Khera *et al.*³ study, suggesting some geographical or diagnostic similarities.

Male dominance in hemoglobinopathies of our study and Abbasi *et al.*⁴ study shows a higher proportion of males affected, suggesting a potential genetic or environmental factor favoring male prevalence. Our study aligns closely with Abbasi *et al.*⁴ study, reinforcing a trend where males are slightly more affected by hemoglobinopathies.

Table 3: Comparison of RBC indices of Beta thalassemia minor with other studies

RBC indices for Beta Thalassemia Minor	Year of Study	HB (gm/dL) mean	MCV (fL) mean	MCH (pg) mean	MCHC (g/dL) mean	HCT (%) mean	RDW (%) mean
Present study	2025	10.33	61.7	21.85	32.71	32.51	19.65
Khera <i>et al</i> ³	2014	9.3	62.7	19.56	31.4	-	19.5
Shrivastav <i>et al</i> ⁵	2013	10.4	62.1	19.4	30.3	-	18.7

Table 3 shows that the present study reports higher hemoglobin levels compared to Khera *et al.*,³ but is similar to Shrivastav *et al.*⁵ Where MCV, MCH, MCHC, and RDW values are comparable across all studies, with minor variations.

LIMITATIONS

Small sample size: The study was limited to only 100 cases.
Short duration: Data collection occurred over a very brief period of three months, between May 2023 and July 2023.

Geographic constraint: The research was conducted as a single-center study at a tertiary care hospital in Ahmedabad, which may not represent the broader population.

Methodological scope: While the study emphasizes HPLC and RBC indices, it notes that definitive identification of certain variants can only be achieved through DNA analysis or amino acid sequencing, which were not utilized in this specific methodology.

CONCLUSION

Automated cell counter-based parameters provide excellent hematological data and play a crucial role in the screening of thalassemias and other hemoglobinopathies. All red cell indices provide essential support to the diagnosis and monitoring of hematological disorders. In this study, we have analyzed the usefulness of HPLC along with other hematological parameters to detect hemoglobin variants. HPLC was helpful in detecting double heterozygous hemoglobinopathies, also. Hematological parameters, along with HPLC findings, aid in detecting and quantifying hemoglobin variants. HPLC forms a rapid, accurate, and reproducible tool for the detection of hemoglobin variants.

Author contributions: SP: Conceived and designed the study, led data acquisition, analysis, and interpretation, drafted the initial manuscript, and coordinated contributions from co-authors; SD: Contributed substantially in studying, designing, and data acquisition, participated in data analysis and interpretation, and critically reviewed and revised the manuscript for important intellectual content; BP: Provided significant intellectual input (e.g., methodology refinement, statistical analysis, and subject-matter expertise), assisted with interpretation of results, and reviewed and revised the manuscript critically. All authors approved the final version of the manuscript and agreed to be accountable for the integrity, accuracy, their specific contributions and the overall work.

Ethical approval: Institutional Review Board approval is not required as this is a prospective study carried out in the Civil Hospital, Ahmedabad.

Declaration of patient consent: Patient's consent not required as the patient's identity is not disclosed or compromised.

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