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Research Article

Spectrum Of Haemoglobinopathies Performed on HPLC From Samples Received at A Tertiary Centre of Western Rajasthan

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ABSTRACT

This study investigates the spectrum of hemoglobinopathies detected by high-performance liquid chromatography (HPLC) in samples received at a tertiary care center in Western Rajasthan. Over a six-month period, 101 patients with suspected hemoglobin disorders were evaluated for demographic, clinical, and hematological characteristics. Beta thalassemia trait emerged as the predominant abnormality, with a notable female predominance likely due to antenatal screening. Anemia was the most common clinical presentation. The majority of samples showed normal hemoglobin patterns, but a significant minority revealed diverse hemoglobin variants. The findings highlight the utility of HPLC for early and accurate detection of hemoglobinopathies and underscore the need for expanded screening and preventive strategies to effectively manage these inherited disorders in high-prevalence regions.

Keywords*: Hemoglobinopathies, HPLC, global public, High-performance liquid chromatography (HPLC).*

INTRODUCTION

Hemoglobinopathies represent a significant category of inherited disorders that profoundly impact global public health[1][2], with their prevalence particularly elevated in regions such as the Middle East, Southeast Asia, and the Indian subcontinent. According to World Health Organization estimates, approximately 269 million individuals worldwide are carriers of various haemoglobin disorders, and each year, about 300,000 infants are born with severe haemoglobinopathies—the majority in developing nations. In India and neighboring countries, these disorders comprise a major health challenge [3], with the burden manifesting differently across regions due to genetic diversity, migration patterns, and sociocultural practices like endogamy and consanguinity [4].

Among the haemoglobinopathies, thalassemias and variant hemoglobins such as E, S, and D are especially prevalent in the Indian population [5]. For instance, β-thalassemia major and hemoglobin E variants frequently occur in eastern and northeastern parts of India, while sickle cell disease predominates in central regions and among scheduled tribes. The clinical impact of these conditions includes chronic anemia, growth retardation, complications related to transfusions, and considerable psychosocial morbidity, underscoring the necessity for early diagnosis and intervention.

High-performance liquid chromatography (HPLC) [7, 8] has emerged as a cornerstone technology for the identification and characterization of hemoglobin variants due to its speed, precision, and reproducibility. Cation-exchange HPLC, in particular, allows for automated, high-throughput screening, facilitating routine evaluation and monitoring in tertiary care settings [9, 10]. Despite the availability of diagnostic modalities, epidemiological data remain sparse in some regions, impeding optimal resource allocation and targeted public health interventions. This study investigates the spectrum of hemoglobinopathies among patients evaluated by HPLC at a tertiary medical center in western Rajasthan, aiming to delineate prevalence patterns and inform both clinical practice and regional policy-making.

Literature Review

Contemporary investigations underscore the genetic and molecular complexity of hemoglobinopathies and thalassemias. Increasingly sophisticated diagnostic modalities such as HPLC, alongside advances in molecular biology, are enhancing

the precision of classification, prognostication, and management for affected individuals. Understanding the overlapping mechanisms and phenotypic variability remains crucial for effective clinical and laboratory evaluation.

Human haemoglobin is a tetrameric protein, primarily composed of two alpha and two beta globin chains [1p], each with an associated heme group that enables oxygen transport. During development, seven different globin chains are synthesized, including embryonic hemoglobins such as Hb Gower 1, Hb Gower 2, Hb Portland 1, and Hb Portland 2, which are prevalent in early fetal life [3p]. At birth, fetal hemoglobin (HbF, $\alpha 2\gamma 2$) predominates, whereas in adulthood, HbA ($\alpha 2\beta 2$) becomes the major form, with smaller portions of HbA2 ($\alpha 2\delta 2$) and residual HbF, attaining stable adult levels by 6–12 months of age. [5p]

Hemoglobinopathies are primarily caused by mutations that alter the amino acid sequence of globin chains, often resulting from single-nucleotide substitutions [2p]. Most hemoglobin variants show normal biosynthetic pathways except for complex structural changes, as seen with fusion genes (e.g., Hb Lepore [7][8]) or chain extension mutants (e.g., Hb Constant Spring [39]) [4p]. In contrast, thalassemias are disorders of globin synthesis, arising from deletions or mutations that lead to an imbalance in globin chain production without necessarily altering their primary structure. Deletions are especially significant in α-thalassemia, while β-thalassemia frequently involves point mutations affecting gene transcription, RNA processing, or premature termination codonsv[6p].

High-performance liquid chromatography (HPLC), particularly using cation-exchange columns, has revolutionized the detection and quantification of hemoglobin variants due to its precision and reproducibility[7p]. Specific retention times allow for differentiation between common variants—such as HbA (1.93–3.10 min), HbA2 (3.30–3.90 min), Hb D Punjab (3.90–4.30 min), and HbS (4.30–4.70 min)—thus enhancing diagnostic confidence in complex cases[1p, 2p].

The clinical spectrum of thalassemias ranges from asymptomatic carriers to individuals with severe anemia, microcytosis, reticulocytosis, and splenomegaly, reflecting the degree of genetic impairment [6p]. Deletion of a single α-globin gene produces a clinically silent state, whereas loss of three genes leads to HbH disease, characterized by significant hemolytic anemia [8p]. Similarly, β-thalassemia presents variably from traits with mild anemia to major forms with transfusion-dependent anemia, depending on the underlying mutation's impact on globin synthesis [9p].

Beyond simple structural variants, numerous molecular mechanisms contribute to hemoglobinopathies and thalassemias, including promoter mutations, splicing defects, and mRNA instability [4p] [7p]. For example, mutations in the β-globin gene may activate cryptic splice sites or destabilize mRNA, as seen in HbE and certain β-thalassemia alleles. Silent nucleotide substitutions—those that do not change the amino acid sequence—can nevertheless affect RNA processing or stability, producing the thalassemic phenotype. Additionally, complex crossover events, such as those creating Hb Lepore or Constant Spring [8p], and hyper-unstable hemoglobins (e.g., Hb Indianapolis, Hb Quong Sze) further blur the distinction between hemoglobinopathies and thalassemia syndromes.

Some structural hemoglobin variants, such as Hb Lepore and Hb Constant Spring, inherently decrease globin chain stability or synthesis, leading to a clinical picture indistinguishable from thalassemia. Fusion chains (as in Hb Lepore) or chain extension mutations (as in Hb Constant Spring) provide mechanistic insights into the intersection of structural and synthetic globin defects [8p]. Mutations affecting splicing or mRNA stability, as seen with HbE and Hb Knossos, exemplify the intricate relationship between the genotype and resulting clinical manifestations.

Recent molecular and biochemical studies have increased understanding of the diverse ways in which globin gene mutations produce disease. For instance, evidence suggests that structural changes resulting in hyper-instability (e.g., Hb Indianapolis) or aberrant splicing (e.g., HbE, Hb Knossos) are important contributors to the thalassemic phenotype. Intensive study of variant hemoglobin mRNAs has revealed that secondary and tertiary structural changes can underlie genetic instability and deficient protein production, with clinical implications for diagnostics and patient management. [4p][7p]

MATERIALS AND METHODS

Study Design and Population

The observational study was conducted at Dr. Sampurnanand Medical College, Jodhpur, spanning six months including five months of patient recruitment and one month for analysis and documentation. Blood samples were collected from hospital patients suspected of hemoglobinopathies based on clinical history or hematological investigation. Institutional ethical clearance was obtained prior to initiation.

- **Inclusion criteria:** All samples received for suspected hemoglobinopathy analyzed via HPLC.
- **Exclusion criteria:** Hemolyzed samples, volume less than 2 mL, or collected in non-EDTA vials.

A total of 101 samples meeting inclusion criteria were processed and analysed using Cation Exchange High-Performance Liquid Chromatography (CE-HPLC).

Procedure and Laboratory Analysis
Samples were analyzed using the BIORAD 'VARIANT' HPLC system. Complete blood counts were performed using Sysmex automated cell counters, and samples were preserved at 4–8°C until analysis. Data on clinical features, transfusion history, age, and gender were prospectively recorded. HPLC chromatograms were interpreted using manufacturer-defined retention time windows and peak integration.

Sample size was determined using prevalence from literature (P=31%), with the formula:
$N=Z^2 P (100-P) / E^2$
Where, Z=1.96, E=10

In HPLC analyzer, on each run, one calibrator and two controls with one blank were added initially. Ranges outside the acceptable area (between 1-3 million) were rejected. All the data regarding clinical history, history of blood transfusion were recorded and chromatogram results of samples printed. Specific defined windows from manufacturer for specific retention time (the time taken from the sample injection up to the apex of elution peak) and integrated peaks were accordingly assigned.

RESULTS
Demographic Profile

- **Age:** Most patients (37.6%) were ≤10 years old; 30.7% were 21–30 years; mean age was 17.99 years.

Table 1 Age wise distribution

Age (yrs)	No. of patients	Percentage
≤10	38	37.62
11-20	14	13.86
21-30	31	30.69
31-40	10	9.90
41-50	8	7.92
Total	101	100.00

- Gender: Females predominated (69.3%), attributed to routine antenatal HPLC screening.

Table 2 Gender wise distribution

Gender	No. of patients	Percentage
Male	31	30.69
Female	70	69.31
Total	101	100.00

- Clinical: Anemia was the commonest presentation (82.2%), followed by anemia with hepatosplenomegaly (9.9%).

Table 3 Distribution of clinical features

Clinical details	No. of patients	Percentage
Anemia	83	82.18
Anemia+HM	1	0.99
Anemia+HSM	10	9.90
Anemia with jaundice	1	0.99
Anemia with spleenomegaly	2	1.98
For screening	4	3.96
Total	101	100.00

Hematological Parameters

Table 4 Distribution of mean HPLC variables

Variables	Descriptive analysis		
	Mean	SD	Median
Hb	7.23	2.58	7.3
Hct.	25.28	8.5	26.6
RDW/cv	22.66	7.39	20.8
RDW/sd	49.16	17.43	45.6
HbA2	2.93	1.24	2.5
HbF	4.34	16.06	0.6

According to above table, total patients of mean HPCL variables are as follows:-

- The mean hemoglobin is 7.23 and median is 7.3.
- The mean haemotcrit is 25.28 and median is 26.6.

- The mean RDW/cv is 22.66 and median 20.8.
- The mean RDW/sd is 49.16 and median is 45.6
- The mean HbA2 is 2.93 and median is 2.5
- The mean HbF is 4.34 and median is 0.6

Diagnostic Spectrum

Table 5 Distribution of Diagnosis

Diagnosis	No. of patients	Percentage
Beta thalassemia major	3	2.97%
Beta thalassemia trait	15	14.85%
Hb D Punjab	2	1.98%
Hb Q- India heterogeneous	1	0.99%
sickle cell trait with hereditary persistent of foetal haemoglobin	1	0.99%
Normal	79	78.22%
Total	101	100.00

Above table depicts out of total 101 patients, most common 15 (14.85%) diagnosis were found in beta thalassemia trait and 79 (78.22%) patients were normal diagnosis in this study.

Table 6 Distribution of age group with beta thalassemia trait patients

Age (yrs)	No. of patients	Percentage
≤10	2	13.33
11-20	1	6.67
21-30	6	40.00
31-40	2	13.33
41-50	4	26.67
Total	15	100.00

As per the above table, out of total 15 beta thalassemia trait patients, most common 6 (40%) patients were in the age group of 21-30 years and second most common 4 (26.67%) patients were in the age group of 41-50 years.

DISCUSSION

Anemia, defined by reduced hemoglobin concentration, is a multifactorial condition influenced by nutritional deficiencies, chronic blood loss, infections, and hereditary disorders such as hemoglobinopathies. The present study highlights the significant burden of genetic hemoglobin disorders—primarily thalassemias and structural hemoglobin variants—in patients assessed by HPLC at a tertiary care facility in western Rajasthan. These inherited conditions represent a major public health challenge due to their autosomal recessive inheritance, clinical morbidity, and social implications.

Hemoglobinopathies and thalassemias mainly affect the globin chains of the hemoglobin molecule, with alpha (α) and beta (β) thalassemia being the most prevalent globally[9][10]. Historically, these disorders were confined to certain geographical regions, ethnicities, castes, and tribal populations due to endogamy and consanguineous marriage practices, which promoted the persistence of pathogenic alleles in isolated communities. However, increased migration, urbanization, and inter-caste marriages have contributed to a wider distribution of these disorders across populations and regions. This demographic shift has important implications for public health planning, requiring broader awareness and diagnostic capacity in diverse healthcare settings.

Within the Indian subcontinent, β-thalassemia trait emerges as the most common hemoglobinopathy, consistent with earlier reports. In patients with β-thalassemia trait, elevated levels of Hemoglobin A2 (HbA2) between 3.5% and 9%, along with laboratory signs of microcytosis, low mean corpuscular volume (MCV), low mean corpuscular hemoglobin (MCH), and raised red blood cell (RBC) counts, serve as clinical markers aiding diagnosis. The presence of raised fetal hemoglobin (HbF) levels above 90% characterizes β-thalassemia major, while intermediate levels (5-15%) may indicate delta-beta thalassemia traits or hereditary persistence of fetal hemoglobin (HPFH). Such hematological parameters are invaluable in routine screening, particularly where confirmatory molecular diagnostics may not be widely available.

The demographic data from this study reflect a prominent pediatric predominance—nearly 38% of patients were aged ≤10 years—mirroring findings by Singh J et al.[11] and underscoring the early onset and diagnosis typical of severe hemoglobinopathies. The second most common age group was 21-30 years, representing individuals who might be carriers or have milder forms that manifest or are detected later. This middle-aged cohort's presence confirms the chronic and lifelong nature of these disorders, highlighting the need for sustained healthcare support.

Gender-wise, a marked female predominance (69%) was noted, consistent with other studies such as those by Rao S et al.[6] and Bajaj P[12] et al. This skew is likely attributable to the referral bias inherent in routine antenatal screening programs that preferentially test pregnant women for hemoglobinopathies to prevent transmission. While this gender disparity may reflect healthcare access patterns rather than disease prevalence, it nonetheless emphasizes the importance of integrating screening into maternal and child health services.

Clinically, the overwhelming majority of patients (82%) presented with anemia, reinforcing the cardinal symptom connecting hemoglobinopathies and thalassemias. The next common presentation was anemia with hepatosplenomegaly (9.9%), a classic feature in more severe disease due to extramedullary hematopoiesis. Comparison with other studies (Atla B et al. [13] and Khatua B et al. [14]) illustrates variation in clinical manifestations arising from different genotypes, including symptoms such as fever, vaso-occlusive crises, dactylitis, seizures, and growth retardation, supported by the genotype-phenotype heterogeneity intrinsic to hemoglobinopathies.

Regarding diagnostic outcomes, approximately 22% of samples in this study showed abnormal hemoglobin fractions on HPLC. This prevalence aligns closely with comparable regional studies by Bajaj P et al. [12], confirming HPLC as a reliable screening tool providing swift and accurate identification of hemoglobin variants. The predominance of beta thalassemia trait (14.85%) as the most frequent diagnosis corroborates findings from multiple Indian cohorts, including those by Rao S [6] and Chandrashekhar V [15]. The observed prevalence of beta thalassemia major (2.97%) also aligns with established epidemiological data, though some studies like Hosseini S [16] report higher incidences, suggesting regional variation and possible referral biases affecting case detection.

The significant percentage of patients with normal HPLC findings (78.22%) highlights several considerations. Firstly, anemia in many individuals may arise from nutritional or inflammatory causes rather than inherited hemoglobin disorders, underscoring the necessity of comprehensive differential diagnosis. Secondly, some hemoglobinopathies or rare variants might escape detection by HPLC alone, emphasizing the continued need for complementary diagnostic modalities like electrophoresis or molecular genetic testing in select cases.

The study's findings confirm the pivotal role of early detection and diagnosis in managing hemoglobinopathies. Timely identification of carriers through programs such as antenatal screening can facilitate genetic counseling and reduce the incidence of severe homozygous conditions through informed reproductive choices. Moreover, diagnosis enables appropriate clinical management, improving quality of life and reducing complications such as iron overload and organ damage.

Given the social and economic burden posed by these disorders, especially in low- and middle-income countries, integrating HPLC-based screening within regional healthcare infrastructure is crucial. Public health initiatives should prioritize awareness generation, capacity building, and population-level screening strategies adapted to regional genetic profiles and socio-cultural dynamics.

CONCLUSION

This study highlights the significant prevalence and diverse spectrum of hemoglobinopathies encountered in patients screened by HPLC at a tertiary care center in Western Rajasthan. Beta thalassemia trait emerged as the most common abnormality, predominantly affecting young adults and children, with a notable female predominance likely influenced by antenatal screening programs. The high proportion of anemia among patients underscores the clinical importance of considering inherited hemoglobin disorders in differential diagnoses.

The findings reaffirm the utility of cation-exchange HPLC as a rapid, reliable, and effective diagnostic tool for early detection and characterization of hemoglobin variants and thalassemias in routine clinical practice. Early diagnosis facilitates timely intervention, genetic counseling, and implementation of preventive strategies, contributing to improved patient outcomes and reducing the disease burden at the population level.

Given the evolving demographic patterns caused by migration and changing socio-cultural practices, expanded screening and awareness campaigns tailored to regional epidemiology are imperative. Strengthening diagnostic infrastructure and promoting interdisciplinary approaches will be key to managing hemoglobinopathies effectively in India and similar high-prevalence settings. Future studies with larger cohorts and molecular analyses may further elucidate genotype-phenotype correlations and assist in refining diagnostic and therapeutic protocols.

In conclusion, this study provides valuable epidemiological insight into hemoglobinopathies in the western Rajasthan, emphasizing the critical need for enhanced screening, diagnosis, and patient management to mitigate the substantial health and social impacts of these inherited disorders.

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