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A Study of Hemoglobinopathies Using High-Performance Liquid Chromatography at a Tertiary Care Hospital in Nizamabad: A Retrospective Cross-Sectional Study.

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Abstract

Background: Hemoglobinopathies are common inherited disorders caused by quantitative or structural abnormalities of globin chains. Their clinical expression ranges from asymptomatic carrier states to severe transfusion-dependent anemia. Cation-exchange high-performance liquid chromatography (HPLC) provides rapid separation and quantification of hemoglobin fractions. ***Objectives:*** To describe the spectrum of hemoglobinopathies detected by HPLC and examine their association with hematological indices among patients evaluated at a tertiary care hospital in Nizamabad. ***Methods:*** This retrospective cross-sectional study included 213 patients aged below 60 years who underwent hemoglobin analysis between January 2024 and August 2025. Complete blood counts and red-cell indices were obtained using an automated hematology analyzer. Hemoglobin fractions were analyzed with the Bio-Rad D-10 cation-exchange HPLC system. Frequencies, percentages, means, standard deviations, and the correlation between HbA2 and mean corpuscular volume were calculated. ***Results:*** Normal hemoglobin profiles were observed in 157 (73.71%) patients, whereas 56 (26.29%) had abnormal profiles. Among abnormal cases, beta-thalassemia trait was most frequent (28, 50.00%), followed by sickle cell trait (7, 12.50%), beta-thalassemia major (5, 8.93%), homozygous sickle cell anemia (5, 8.93%), delta-beta-thalassemia (5, 8.93%), and HbS-beta-thalassemia (3, 5.36%). HPFH heterozygosity, HbH disease, and HbQ India trait were identified in one patient each. Mean hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin among abnormal cases were 8.2 ± 2.1 g/dL, 61.3 ± 8.4 fL, and 19.2 ± 3.1 pg, respectively. HbA2 correlated inversely with mean corpuscular volume. ***Conclusion:*** HPLC identified a broad spectrum of common, severe, compound, and rare hemoglobin patterns. The high proportion of carrier states supports integrated hematological assessment, confirmatory testing for ambiguous profiles, family screening, and genetic counseling.

Keywords: Beta-thalassemia; cation-exchange HPLC; hemoglobin variants; hemoglobinopathies; sickle cell disease; screening

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INTRODUCTION

Hemoglobinopathies are inherited disorders produced by mutations that alter the synthesis or structure of globin chains. They include thalassemia syndromes, in which globin-chain production is reduced or absent, and structural variants such as hemoglobin S, E, D, and C. Clinical expression ranges from clinically silent heterozygous states to chronic hemolytic anemia, vaso-occlusive disease, organ damage, growth impairment, and lifelong transfusion dependence. Because carriers are often asymptomatic, laboratory identification has a central role in case detection, reproductive counseling, and prevention of severe disease in offspring [1,2].

The burden of hemoglobin disorders is substantial in India, where population diversity, endogamy, consanguinity, and geographically clustered mutations produce marked regional variation. Beta-thalassemia trait and sickle cell trait are important carrier states, while beta-thalassemia major, sickle cell disease, and compound heterozygous conditions contribute to recurrent hospitalization and sustained requirements for transfusion, chelation, and specialist care [2,3]. Screening studies from different Indian regions have documented wide spectra of common and uncommon variants, emphasizing that local data are necessary for planning diagnostic services and targeted prevention programs [4-7]. Telangana lies within a region where both thalassemic and sickling disorders are encountered, yet hospital-based data from Nizamabad remain limited.

Traditional alkaline electrophoresis is useful for separating several hemoglobin fractions, but overlapping migration patterns, limited quantification, and operator-dependent interpretation can create diagnostic uncertainty. Cation-exchange high-performance liquid chromatography (CE-HPLC) separates hemoglobin fractions according to ionic interactions and reports characteristic retention windows with quantitative peak areas. The technique offers rapid processing, reproducibility, and objective measurement of HbA2 and HbF, which are particularly important for recognizing beta-thalassemia trait and differentiating thalassemic microcytosis from other causes [8-10]. Nevertheless, HPLC patterns require interpretation alongside age, transfusion history, red-cell indices, clinical features, and, when indicated, a second analytical or molecular method because co-eluting or rare variants can generate ambiguous profiles [8,9].

Early identification of carrier states enables partner testing, cascade family screening, antenatal diagnosis, and informed reproductive decisions. Indian antenatal screening programs have shown that systematic detection followed by counseling can identify couples at risk of having children with severe homozygous or compound heterozygous disorders [11,12]. A regional description of HPLC patterns can therefore support both clinical diagnosis and public-health planning.

The present study aimed to determine the proportion and distribution of hemoglobinopathies detected by CE-HPLC among patients evaluated at a tertiary care hospital in Nizamabad, to describe the demographic profile of affected individuals, and to examine hematological indices and the relationship between HbA2 and mean corpuscular volume in abnormal cases.

MATERIALS AND METHODS

Study design and setting: This retrospective, cross-sectional descriptive study was conducted in the Department of Biochemistry, Government General Hospital, Nizamabad, Telangana, India. Laboratory records generated from January 2024 through August 2025 were reviewed. The manuscript was prepared with reference to the Strengthening the Reporting of Observational Studies in Epidemiology recommendations [13].

Study population: The analysis included 213 patients of either sex, ranging from infancy to below 60 years of age, who underwent hemoglobin fraction analysis during the study period. Referral indications comprised unexplained or refractory microcytic hypochromic anemia, inadequate response to iron therapy, suspected hemolytic disease indicated by features such as splenomegaly, recurrent jaundice, or bone pain crises, antenatal or premarital screening, and severe anemia or failure to thrive in infants and children. Patients who had received a blood transfusion during the preceding three months were excluded from definitive variant classification because donor erythrocytes can mask or alter hemoglobin fractions. All eligible records with corresponding hematological and chromatographic data were included.

Sample collection and hematological analysis: Peripheral venous blood, collected in ethylenediaminetetraacetic acid vacutainers, was used for complete blood count and hemoglobin analysis. Hemoglobin concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH) were measured using an automated five-part hematology analyzer. Hematological indices were reviewed with the chromatographic pattern because reduced MCV and MCH strengthen the interpretation of thalassemic phenotypes, whereas iron deficiency and other microcytic disorders can influence screening parameters [9,10].

HPLC procedure and interpretation: Hemoglobin fraction analysis was performed using the Bio-Rad D-10 automated analyzer based on cation-exchange HPLC. Samples were diluted and hemolyzed automatically, injected into an ion-exchange cartridge, and separated using a programmed buffer gradient. Eluted fractions were detected photometrically at 415 nm, and the instrument generated chromatograms with retention times and percentages of hemoglobin fractions. Interpretation followed established chromatographic principles [4,8,14]. A normal adult profile was defined as HbA greater than 95%, HbA2 2.0-4.0%, and HbF below 1%. Beta-thalassemia trait was assigned when HbA2 exceeded 4.0% with compatible microcytosis and hypochromia. Beta-thalassemia major, sickle cell trait, homozygous sickle cell anemia, HbS-beta-thalassemia, delta-beta-thalassemia, hereditary persistence of fetal hemoglobin, HbH disease, and HbQ India trait were classified from characteristic fraction patterns, retention windows, and hematological findings. Rare or potentially co-eluting patterns were regarded as presumptive HPLC identifications.

Statistical analysis: Data were entered in Microsoft Excel and analyzed descriptively. Categorical variables are presented as frequencies and percentages; continuous variables are summarized as mean ± standard deviation. Pearson correlation was used to assess the relationship between HbA2 and MCV. A two-sided p value below 0.05 was considered statistically significant.

Ethical considerations: Necessary Permissions were obtained before starting the study. The informed-consent or consent-waiver status must be completed according to the committee approval. Patient identifiers were removed before analysis.

RESULTS

During the study period, 213 eligible patient records were analyzed. A normal HPLC profile was present in 157 (73.71%) patients, while 56 (26.29%) had an abnormal hemoglobin pattern. The study population included 117 (54.93%) males and 96 (45.07%) females. Among the abnormal cases, 34 (60.71%) were male and 22 (39.29%) were female. The corresponding distribution of normal and abnormal profiles by sex is presented in Table 1.

Table 1. Hemoglobin profile according to sex

Sex	Overall, n (%)	Normal profile, n (%)	Abnormal profile, n (%)
Male	117 (54.93)	83 (52.87)	34 (60.71)
Female	96 (45.07)	74 (47.13)	22 (39.29)
Total	**213 (100.00)**	**157 (100.00)**	**56 (100.00)**

Participants ranged from infancy to below 60 years. Children aged 1-12 years constituted the largest overall age group, accounting for 84 (39.44%) participants, followed by infants in 48 (22.54%) and adolescents in 41 (19.25%). Among the 56 abnormal profiles, 21 (37.50%) occurred in children, 14 (25.00%) in infants, and 12 (21.43%) in adolescents. Thus, 35 of the 56 abnormal cases (62.50%) were detected before 13 years of age. The complete age distribution is shown in Table 2.

Table 2. Age distribution of the overall sample and HPLC profile groups

Age group	Overall, n (%)	Normal profile, n (%)	Abnormal profile, n (%)
Infants (<1 year)	48 (22.54)	34 (21.66)	14 (25.00)
Children (1-12 years)	84 (39.44)	63 (40.13)	21 (37.50)
Adolescents (13-19 years)	41 (19.25)	29 (18.47)	12 (21.43)
Adults (20-40 years)	32 (15.02)	25 (15.92)	7 (12.50)
Older adults (>40 years)	8 (3.76)	6 (3.82)	2 (3.57)
Total	**213 (100.00)**	**157 (100.00)**	**56 (100.00)**

Beta-thalassemia trait was the predominant abnormality, identified in 28 patients and representing 50.00% of abnormal profiles and 13.15% of all patients. Sickle cell trait was identified in seven patients. Beta-thalassemia major, homozygous sickle cell anemia, and delta-beta-thalassemia were each observed in five patients, whereas HbS-beta-thalassemia was present in three. Three rare patterns were detected: one case each of heterozygous hereditary persistence of fetal hemoglobin, HbH disease, and HbQ India trait. Carrier states comprising beta-thalassemia trait and sickle cell trait together accounted for 35 (62.50%) abnormal profiles. Severe disease patterns comprising beta-thalassemia major, homozygous sickle cell anemia, and HbS-beta-thalassemia accounted for 13 (23.21%) abnormal profiles. Variant frequencies are detailed in Table 3.

Table 3. Distribution of abnormal hemoglobin patterns detected by HPLC

Hemoglobin pattern	n	% of abnormal cases	% of total sample
Beta-thalassemia trait	28	50.00	13.15
Sickle cell trait	7	12.50	3.29
Beta-thalassemia major	5	8.93	2.35
Homozygous sickle cell anemia (HbSS)	5	8.93	2.35
Delta-beta-thalassemia, heterozygous	5	8.93	2.35
HbS-beta-thalassemia	3	5.36	1.41
HPFH, heterozygous	1	1.79	0.47
HbH disease	1	1.79	0.47

Hemoglobin pattern	n	% of abnormal cases	% of total sample
HbQ India trait	1	1.79	0.47
Total abnormal profiles	**56**	**100.00**	**26.29**

Abnormal cases showed pronounced anemia, microcytosis, and hypochromia. Mean hemoglobin concentration was 8.2 ± 2.1 g/dL, mean MCV was 61.3 ± 8.4 fL, and mean MCH was 19.2 ± 3.1 pg. The relationship between HbA2 and MCV was moderately strong and inverse ($r = -0.68$, $p < 0.001$), indicating that higher HbA2 values were associated with lower MCV. Patients classified as beta-thalassemia trait clustered predominantly above the study HbA2 threshold and within the microcytic range. Hematological findings and the correlation result are summarized in Table 4.

Table 4. Hematological findings among abnormal HPLC profiles

Measure	Result	Reference or interpretive range
Hemoglobin	8.2 ± 2.1 g/dL	12-16 g/dL
Mean corpuscular volume	61.3 ± 8.4 fL	80-96 fL
Mean corpuscular hemoglobin	19.2 ± 3.1 pg	27-32 pg
HbA2-MCV correlation	$r = -0.68$; $p < 0.001$	Inverse association

DISCUSSION

The present study demonstrated abnormal HPLC patterns in 26.29% of referred patients, with beta-thalassemia trait accounting for half of all abnormalities. This proportion reflects a selected hospital population evaluated for anemia, suspected hemolysis, or screening and should not be interpreted as community prevalence. Nevertheless, the predominance of beta-thalassemia trait is consistent with Indian HPLC series in which carrier states constitute a major share of detected abnormalities [4-7]. The finding has practical significance because carriers commonly have mild microcytosis and can be treated repeatedly for presumed iron deficiency unless HbA2 and the complete red-cell profile are assessed.

Sickle cell trait was the second most frequent pattern, while homozygous sickle cell anemia and HbS-beta-thalassemia together formed a clinically important subgroup. The coexistence of thalassemic and sickling disorders in the same referral population supports the need for diagnostic pathways that recognize compound heterozygosity rather than classifying all prominent HbS peaks as a single disease entity. CE-HPLC provides rapid quantitative separation and characteristic retention windows, but interpretation requires clinical information, transfusion history, HbA2, HbF, and red-cell indices [8,14]. Molecular testing or an independent analytical technique remains important when peaks co-elute, percentages are atypical, or a rare variant is suspected.

The mean MCV and MCH among abnormal cases confirmed marked microcytosis and hypochromia. The inverse correlation between HbA2 and MCV further supports the diagnostic contribution of combining chromatographic fractions with routine hematology. Previous work has shown that HPLC can identify most high-HbA2 beta-thalassemia carriers even when iron deficiency coexists, although iron status can modify HbA2 values and complicate borderline profiles [9,10]. Accordingly, a normal or borderline HbA2 result should not end evaluation when microcytosis is persistent, family history is suggestive, or iron deficiency has not been adequately assessed.

More than three-fifths of abnormal cases were detected in infants and children. Severe disorders often become clinically evident early, while carrier states can be recognized incidentally during anemia evaluation or family screening. Early diagnosis permits appropriate transfusion planning, avoidance of unnecessary iron therapy, vaccination and prophylactic measures for sickle cell disease, and timely referral for genetic counseling. Indian antenatal programs have demonstrated the feasibility of detecting at-risk couples through staged screening and partner testing [11,12]. Extending similar pathways to family members of affected children could improve case finding in Nizamabad.

Rare patterns, including HPFH, HbH disease, and HbQ India trait, illustrate the broad analytical reach of HPLC but also expose its interpretive limits. Their identification in this study should be regarded as presumptive because molecular confirmation was unavailable. Overall, the findings support CE-HPLC as a valuable first-line platform within a tiered diagnostic strategy that integrates hematological indices, confirmatory testing, family studies, and reproductive counseling.

Limitations

This study was retrospective, single-center, and based on a referral population, limiting estimation of community prevalence and introducing selection bias. Iron studies, clinical severity, treatment history, ethnicity, and family data were

unavailable for uniform analysis. Rare and compound patterns lacked confirmation by electrophoresis, capillary methods, or molecular testing. Age-specific hemoglobin fractions and recent transfusion histories could also influence chromatographic interpretation.

CONCLUSION

Cation-exchange HPLC detected abnormal hemoglobin patterns in approximately one-quarter of referred patients and revealed a heterogeneous spectrum dominated by beta-thalassemia trait. Carrier states were more frequent than severe disorders, while sickle cell disease, HbS-beta-thalassemia, delta-beta-thalassemia, HPFH, HbH disease, and HbQ India expanded the diagnostic range. Marked microcytosis and hypochromia, together with the inverse HbA2-MCV relationship, supported integrated interpretation of chromatographic and hematological findings. HPLC should serve as a first-line method within a tiered diagnostic pathway rather than as an isolated confirmatory test. Regional programs should combine early detection, iron-status assessment, second-method or molecular confirmation of uncertain profiles, family screening, partner testing, and genetic counseling to reduce preventable severe hemoglobin disorders.

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