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HPLC IN CHARACTERISATION OF HEMOGLOBIN PROFILE IN THALASSEMIA SYNDROMES: A CLINICOHEMATOLOGICAL CORRELATION

Pathology

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ABSTRACT

Background: Thalassemias and hemoglobinopathies are the most common inherited disorders of hemoglobin synthesis worldwide, posing significant public health challenges, especially in high-prevalence regions such as India. High Performance Liquid Chromatography (HPLC) has emerged as a powerful diagnostic tool for the identification and quantification of hemoglobin variants, facilitating early and accurate diagnosis critical for disease management and prevention.**Objective:** To evaluate the clinicohematological profile of patients with thalassemia syndromes and assess the usefulness of HPLC in characterising hemoglobin profiles, correlating these with clinical and hematological parameters.**Methods:** This prospective, hospital-based cross-sectional study was conducted over one year in the Department of Pathology, Jhalawar Medical College and Hospital. Eighty-six patients suspected of thalassemia or hemoglobinopathies based on clinical and hematological criteria underwent detailed clinical evaluation and hemoglobin analysis via HPLC. Hematological indices including hemoglobin concentration, RBC indices, reticulocyte count, and peripheral blood film evaluation were recorded. Statistical analysis was performed using SPSS v29.**Results:** Of 86 subjects, 71 (82.6%) were diagnosed with thalassemia syndromes: 42 (48.8%) thalassemia trait, 16 (18.6%) thalassemia major, 8 (9.3%) thalassemia intermedia, and 5 (5.8%) double heterozygous. Female predominance was observed (55.8%). Clinical findings such as pallor, splenomegaly, and hemolytic facies varied significantly across groups, with all thalassemia major and double heterozygous patients showing splenomegaly and need for transfusions. Mean hemoglobin levels ranged from 5.25 g/dL in intermedia to 8.88 g/dL in trait. HPLC revealed elevated HbF in double heterozygous (50.8%) and lowest in trait (2.53%), while HbA2 was highest in intermedia (18.87%) and lowest in major (2.41%). Significant correlations were found between hemoglobin fractions and clinical parameters, validating HPLC as an effective diagnostic modality.**Conclusion:** HPLC provides rapid, accurate, and reproducible detection and quantification of hemoglobin variants, crucial for diagnosing thalassemia syndromes. Its integration with clinical and hematological evaluation enhances diagnostic precision, enabling timely management and genetic counselling. Screening programs incorporating HPLC are essential to reduce the burden of hemoglobinopathies in resource-limited settings.

KEYWORDS

Thalassemia, Hemoglobinopathies, High Performance Liquid Chromatography, Clinicohematological Correlation.

INTRODUCTION

Thalassemias are autosomal recessive disorders characterized by absent or reduced synthesis of one or more globin chains of hemoglobin, leading to clinical presentations ranging from asymptomatic carrier states to severe, transfusion-dependent anemia.[1,2]

High prevalence occurs mainly in the Mediterranean basin, India, and Southeast Asia.[3]

Diagnosis is based on clinical features, hematological indices, and confirmatory tests including high performance liquid chromatography (HPLC), which is now considered a gold standard method for screening and diagnosis due to its sensitivity and specificity.[4]

MATERIALS AND METHODS

Study Design: Prospective, cross-sectional hospital-based study conducted at the Department of Pathology, Jhalawar Medical College and Hospital from July 2023 to July 2024.

Subjects: 86 patients fulfilling inclusion criteria (microcytic hypochromic anemia, clinical suspicion of thalassemia/ hemoglobinopathy, transfusion-dependent cases, antenatal cases and relatives). Exclusions included recent transfusion and other hemoglobinopathies.

Clinical Evaluation: Detailed history and physical examination noting pallor, splenomegaly, hemolytic facies, and transfusion history.

LABORATORY TECHNIQUES:

- Complete blood count (Sysmex XN 1000).
- Peripheral blood smear (Leishman Stain).
- Reticulocyte count (supravital staining).
- Hemoglobin analysis by ion-exchange HPLC using Bio-Rad Variant II system with beta thalassemia short program.

Statistical Analysis: Conducted with SPSS 29; descriptive statistics, correlation analysis (Pearson, Spearman) with significance set at $p<0.05$.

RESULTS

Table 1: Distribution Of Clinical Findings

Clinical Feature	Trait (%)	Intermedia (%)	Major (%)	Double Heterozygous (%)
Pallor	28.6	87.5	100	100
Splenomegaly	0	25	100	60
Hemolytic Facies	0	37.5	43.75	20
Blood Transfusion Required	2.38	100	100	100

Table 2: Mean hematological parameters

Parameter	Normal (Mean ± SD)	Trait (Mean ± SD)	Intermedia (Mean ± SD)	Major (Mean ± SD)	Double Heterozygous (Mean ± SD)
Hb (g/dL)	10.2 ± 1.33	8.88 ± 2.75	5.25 ± 2.21	5.84 ± 2.16	5.74 ± 0.58
MCV (fL)	70.01 ± 11.74	69.49 ± 14.27	67.25 ± 7.03	82.12 ± 11.64	63.08 ± 2.28
MCH (pg)	22.92 ± 4.37	21.48 ± 5.1	20.23 ± 1.58	27.45 ± 4.64	25.8 ± 6.26
MCHC (g/dL)	32.59 ± 1.23	31.12 ± 2.67	30.18 ± 1.07	33.48 ± 4.53	40.62 ± 8.9
RBC (million/µL)	4.5 ± 0.33	4.47 ± 1.68	2.08 ± 0.72	2.12 ± 0.51	2.32 ± 0.51
Reticulocyte %	1.19 ± 0.37	2.3 ± 0.53	3.68 ± 1.49	4.25 ± 1.24	3.3 ± 0.45

Table 3: Mean hemoglobin fractions by diagnosis

Diagnosis	HbF Mean (%) ± SD	HbA Mean (%) ± SD	HbA2 Mean (%) ± SD
Normal	1.3 ± 0	82.72 ± 1.47	2.54 ± 0.35
Double Heterozygous	50.8 ± 6.93	22.56 ± 9.70	14.5 ± 24.15
Thalassemia Intermedia	37.68 ± 14.26	30.55 ± 9.86	18.88 ± 29.46

Thalassemia Major	46.46 ± 21.87	39.19 ± 28.33	2.41 ± 1.14
Thalassemia Trait	2.53 ± 4.87	77.31 ± 16.02	5.84 ± 1.02

CORRELATIONS:

- Significant positive correlation of HbF with MCH in thalassemia major ($p=0.048$).
- Significant negative correlation of HbA2 with splenomegaly in thalassemia trait ($p=0.002$).
- Significant positive correlation of HbA2 with hemoglobin in thalassemia trait ($p=0.016$).
- Significant positive correlation of HbF, HbA2, and HbA with various thalassemic disorders ($p<0.001$).

DISCUSSION

This prospective study highlights the clinicohematological spectrum of thalassemia syndromes in a hospital-based population using HPLC for hemoglobin characterization.[5]

The predominance of thalassemia trait aligns with epidemiological data showing a high carrier rate in India. Clinical manifestations correlated well with severity, with marked splenomegaly and transfusion requirement in thalassemia major and double heterozygous patients. Hematological parameters showed expected trends, with reduced Hb, MCV, and MCH in more severe forms.[6]

HPLC proved an efficient, reproducible diagnostic tool distinguishing normal from various thalassemia subtypes and hemoglobin variants, offering advantages over traditional electrophoresis. The correlations of HbF and HbA2 levels with clinical severity underscore their diagnostic and prognostic relevance. The study supports routine use of HPLC in thalassemia screening programs to facilitate early detection and management, which is critical in countries with high disease burden and limited resources.[7]

Limitations include the small sample size and hospital-based design; larger population studies and longitudinal follow-up are warranted.

CONCLUSION

High performance liquid chromatography is a reliable, rapid, and precise method for screening and diagnosing thalassemia and hemoglobinopathies. Its adoption in clinical practice enables early identification and better management of affected patients and carriers, aiding in disease control efforts in high prevalence regions.

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