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RESEARCH ARTICLE
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"HEMOGLOBINOPATHIES IN CHILDREN OF TRIBAL REGION OF MADHYA PRADESH: CORRELATION OF HEMATOLOGICAL INDICES AND HPLC"

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Abstract

Background: Hemoglobinopathies are among the most common inherited disorders worldwide, particularly prevalent in paediatric populations in certain geographical regions. Early diagnosis using haematological indices and confirmatory techniques such as High Performance Liquid Chromatography (HPLC) is essential for effective disease management and genetic counselling.

Objective: To evaluate the spectrum of hemoglobinopathies in the paediatric age group using hematological indices and to correlate these findings with HPLC results.

Methods: This cross-sectional study was conducted on paediatric patients (aged 0–13 years) suspected of having hemoglobinopathies. Complete blood counts and red cell indices (Hb, RBC count, MCV, MCH, MCHC, RDW) were recorded using automated hematology analysers. Based on suggestive hematological parameters, samples were further analysed using HPLC for definitive diagnosis. Data were statistically analysed to assess the correlation between hematological indices and HPLC findings.

Results: Out of the total samples analysed, a significant proportion showed abnormal hematological indices suggestive of thalassemia traits or other hemoglobin variants. HPLC confirmed diagnosis including β-thalassemia trait, HbE trait, HbS trait, and compound heterozygous states. A strong correlation was found between reduced MCV/MCH values and β-thalassemia trait, while normal indices were seen in some hemoglobin variants. HPLC proved crucial for differentiating between similar phenotypes on hematological grounds.

Conclusion: Hematological indices serve as a useful screening tool for suspected hemoglobinopathies in children, but HPLC remains indispensable for accurate diagnosis and characterization. Early identification is vital for appropriate clinical management and genetic counselling.

Keywords: Hemoglobinopathies, Pediatrics, Hematological Indices, HPLC, Thalassemia, Hemoglobin Variants, Red Cell Indices

INTRODUCTION

Hemoglobinopathies are a group of inherited disorders caused by genetic mutations in the hemoglobin molecule, leading to abnormal hemoglobin production or structure. These conditions are among the most common genetic disorders worldwide and significantly impact pediatric populations. In the context of paediatric age groups, hemoglobinopathies such as sickle cell disease, thalassemia, and other variants of abnormal haemoglobin are critical causes of morbidity and mortality. The spectrum of hemoglobinopathies varies widely, and early diagnosis is crucial for effective management and prevention of complications[1].

Hematological indices, including red blood cell (RBC) count, hemoglobin concentration, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), RDW, Reticulocyte count and nRBC are commonly used in the clinical evaluation of patients with suspected hemoglobinopathies. These indices provide valuable insights into the nature of the disorder, with specific patterns observed in various types of hemoglobinopathies[2].

High-performance liquid chromatography (HPLC) is one such method that has revolutionized the diagnosis and characterization of hemoglobinopathies. HPLC allows for the precise identification and quantification of different hemoglobin variants, making it a gold standard in the diagnosis of these disorders[3].

The use of HPLC in conjunction with traditional hematological indices can significantly enhance diagnostic accuracy. HPLC is highly effective in identifying abnormal hemoglobins such as hemoglobin S (HbS) in sickle cell disease or hemoglobin A2 (HbA2) in beta-thalassemia, which are not easily discernible using basic hematological tests alone[4].

In the pediatric population, hemoglobinopathies present unique challenges in both diagnosis and management. Thalassemia major often requires regular blood transfusions and iron chelation therapy, highlighting the importance of early detection. The role of HPLC in diagnosing these conditions early can lead to better clinical outcomes and the prevention of severe complications[5].

Additionally, early identification of hemoglobinopathies in children allows for genetic counselling, family planning, and the initiation of targeted treatments.

In regions with a high prevalence of hemoglobinopathies, such as Tribal areas of Madhya Pradesh, screening programs using both hematological indices and HPLC can be life-saving[6].

AIM & OBJECTIVE: -

To correlate the hematological indices (Hb, MCV, MCH, RDW, Reticulocytes and nRBC) with HPLC finding for accurate diagnosis of hemoglobinopathies in children.

➢ To determine the prevalence and spectrum of various hemoglobinopathies (such as Thalassemia, Sickle cell anemia, HbE disorders and their combinations) in paediatric age group in Vindhya Region of Madhya Pradesh.

MATERIAL & METHOD

Study Design and Setting:

This is a cross-sectional study conducted to identify and characterize hemoglobinopathies in paediatric patients, utilizing hematological indices and high-performance liquid chromatography (HPLC). The study aims to correlate the findings of hematological indices with the HPLC analysis in children diagnosed with hemoglobinopathies.

Sample Size:200 Patients

Inclusion Criteria:

1. Pediatric patients aged 0-14 years.
2. Clinical suspicion of hemoglobinopathy (such as thalassemia, sickle cell anemia, or other hemoglobin variants).
3. Patients have elevated Reticulocytes and nRBC count.

4. Informed consent obtained from parents or guardians.

Exclusion Criteria:
1. Patients with acute infections or chronic diseases affecting hemoglobin synthesis.
2. Patients who are transfusion-dependent at the time of enrolment.
3. Incomplete haematological data or HPLC analysis.

Sample Collection
Peripheral blood samples were collected from each patient in two separate EDTA tubes. One tube was used for complete blood count (CBC) and hematological indices analysis. The second tube was reserved for High-Performance Liquid Chromatography (HPLC) to assess hemoglobinopathies.

Hematological Indices
Complete blood counts and haematological parameters (Hb, RBC count, MCV, MCH, RDW, Reticulocytes and nRBC) were performed using an automated hematology analyser and Peripheral smear examination.

Peripheral Smear Examination
Peripheral blood smears were prepared and stained with Giemsa stain. Microscopic examination focused on identifying red cell morphology including anisocytosis, poikilocytosis, microcytosis, and hypochromia. Characteristic findings such as sickle cells and target cells were also noted.

High-Performance Liquid Chromatography (HPLC)
HPLC was performed by Bio-Rad D10 analysernto confirm hemoglobinopathy diagnosis. Chromatograms were analysed to determine the type and proportion of hemoglobin variants. Patterns suggestive of conditions like HbSS, HbSC, HbS/β-thalassemia, or β-thalassemia trait were documented.

Statistical Analysis
Data were entered in Microsoft Excel and analyzed using SPSS (version X.X). Continuous variables were expressed as mean ± standard deviation, and categorical variables as frequency and percentages.

RESULTS
The study included a total of 200 paediatric patients, ranging in age from infancy to 14 years. The age group with the highest representation was 6–8 years, comprising 32% (n=64) of the total cases. This was followed by the 9–10 years age group, accounting for 25% (n=50). Children aged 11–14 years made up 19% (n=38) of the study population, while 3–5 years accounted for 18% (n=36). The youngest age group, 0–2 years, represented the smallest proportion, contributing only 6% (n=12) of the cases. Paediatric patients screened for hemoglobinopathies is nearly equal, with a slight female predominance (51% females vs. 49% males).

Among the 200 paediatric patients studied, weakness was the most commonly reported symptom, observed in 55% (n=110) of cases. Pallor was the next most frequent, seen in 41% (n=82) of children, followed by loss of appetite, reported in 37% (n=74). Less common but clinically significant findings included icterus in 16% (n=32) of patients and failure to thrive in 12% (n=24). Splenomegaly with hepatomegaly was identified in 8% (n=16), while edema was the least prevalent symptom, occurring in 6% (n=12).

Table 1 Hematological Indices of patients: -

S.No.	Hematological Indices	Mean ± SD
01	Hemoglobin (g/dL)	8.9 ±2.1
02.	MCV (fL)	72.2 ± 8.7
03.	MCH (pg)	22.1 ± 3.5
04.	RBC Count	3.5 ± 0.87
05.	RDW (%)	18.3 ± 3.2
06.	Reticulocytes	5.330±1.935
07.	nRBC	7.3±5.35

The hematological indices of the pediatric patients revealed significant abnormalities consistent with hemoglobinopathies. The mean hemoglobin level was 8.9 ± 2.1 g/dL, indicating a high prevalence of anemia. MCV (72.2 ± 8.7 fL) and MCH (22.1 ± 3.5 pg) were both reduced, suggestive of microcytic, hypochromic anemia typically seen in thalassemia traits. The RBC count was relatively low (3.5 ± 0.87 million/μL), while the RDW (18.3 ± 3.2%) was elevated, indicating significant anisocytosis. An increased reticulocyte count (5.33 ± 1.94%) and nucleated RBCs (7.3 ± 5.35) further suggest active erythropoiesis, possibly due to chronic hemolysis or marrow compensation in response to anemia.

Table 2: Hematological Indices by Hemoglobinopathy

Hemoglobinopathy	Mean Hb (g/dL)	Mean MCV (fL)	Mean MCH (pg)	Mean RDW (%)
HBS Homozygous (Sickle cell disease)	8.6	92.1	27.4	19.5
Sickle cell & Beta Thalassemia (Double heterozygous)	9.2	75	21.2	20.0
HBS Heterozygous (Sickle cell trait)	11.8	89.5	26.3	15
Beta Thalassemia trait	9.6	73	23.5	12.3
Hb-D trait	12.5	88.9	26.7	12.3
Beta Thalassemia major	7.4	65.7	23.2	19.2
HbE-trait	11.7	84.8	34.9	14.4

The hematological indices varied significantly across different hemoglobinopathies. Patients with Beta Thalassemia major showed the lowest hemoglobin and MCV values, reflecting severe anemia and microcytosis. In contrast, Hb-D trait and HbE trait exhibited higher hemoglobin levels with near normal MCV and MCH. Elevated RDW was most pronounced in sickle cell disease and double heterozygous cases, indicating greater red cell size variability.

Table 3: Reticulocyte and NRBC Distribution

Hemoglobinopathy	Mean Reticulocyte (%)	Reticulocyte Range (%)	Mean NRBC (/100 WBC)	NRBC Range (/100 WBC)
HBS Homozygous (Sickle cell disease)	7.2	3.5-8.0	19.8	6-180
Sickle cell & Beta Thalassemia (Double heterozygous)	4.6	3.5-8.0	6.8	6-18
HBS Heterozygous (Sickle cell trait)	2.2	2-7.5	1.6	1-25
Beta Thalassemia trait	2.2	2.0-7.0	3.2	2-28
Hb-D trait	1.0	1.0-6.0	1.5	1-20
Beta Thalassemia major	8.0	3.0-19	21.0	15-30
HbE-trait	1.2	1.0-6.0	5.0	1-15
Normal Pattern	4.8	1-22	7.2	6-18

Patients with Beta Thalassemia major and HBS homozygous (sickle cell disease) showed the highest mean reticulocyte counts (8.0% and 7.2%, respectively) and elevated NRBC levels, indicating increased marrow activity and stress erythropoiesis. In contrast, traits like Hb-D and HbE had low reticulocyte and NRBC counts, similar to normal patterns. Double heterozygous cases showed intermediate values, reflecting variable disease severity.

The distribution of hemoglobinopathies in this pediatric cohort highlights a substantial burden, with nearly 48% diagnosed with abnormal hemoglobin variants. The most common was sickle cell trait (HbS heterozygous) at 20%, followed by sickle cell disease (HbS homozygous) at 14.5%, reflecting a high carrier frequency and disease prevalence. Beta thalassemia trait accounted for 6.5%, and beta thalassemia major, a severe form requiring transfusions, was seen in 2% of patients. Compound heterozygosity for sickle cell and beta thalassemia was present in 3%, underscoring the clinical complexity in some cases. Less frequent variants included HbE trait (1.5%) and HbD trait (0.5%). Over half the children (52%) showed no detectable hemoglobinopathy, indicating these disorders are common but not universal in this population.

Graph: Hemoglobinopathy Types by Frequency

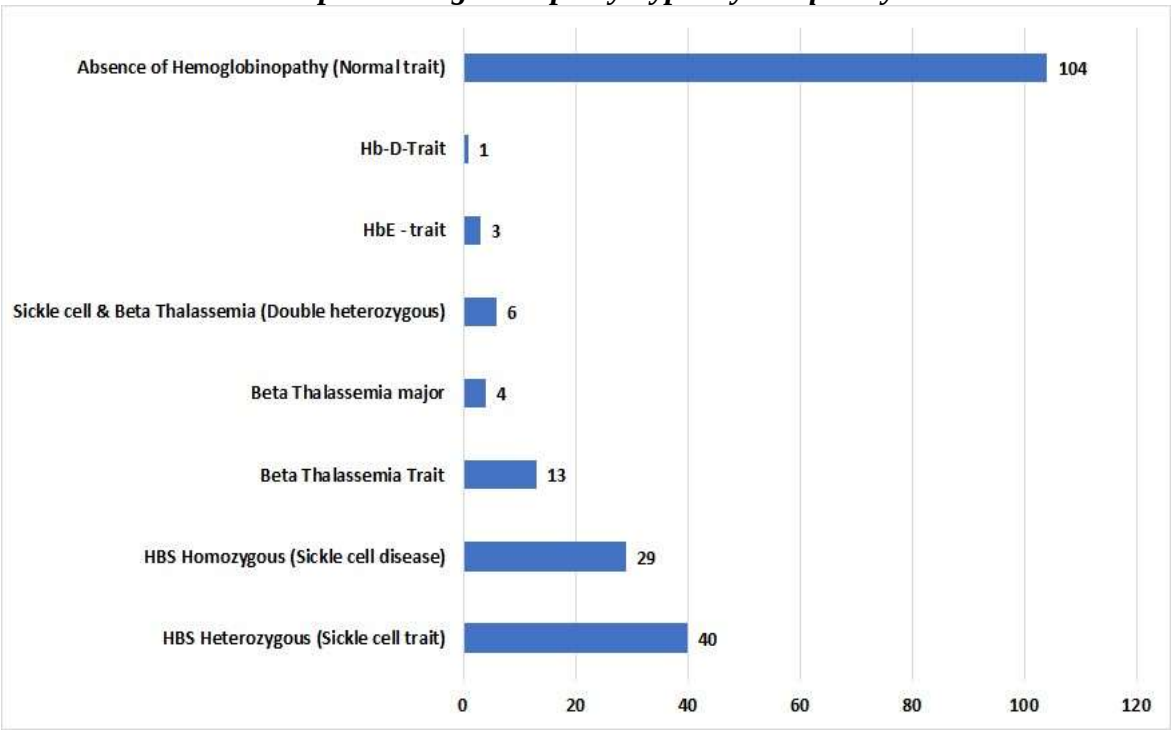


Table 4: MCV and MCH Patterns in Microcytic vs. Normocytic Cases

Peripheral Smear Comment	Mean MCV (fL)	Mean MCH (pg)	Count
Normocytic Normochromic	92.3	27.1	150
Microcytic Hypochromic	72.8	23.2	48
Macrocytic anemia	107	31.5	02
Total	-	-	200

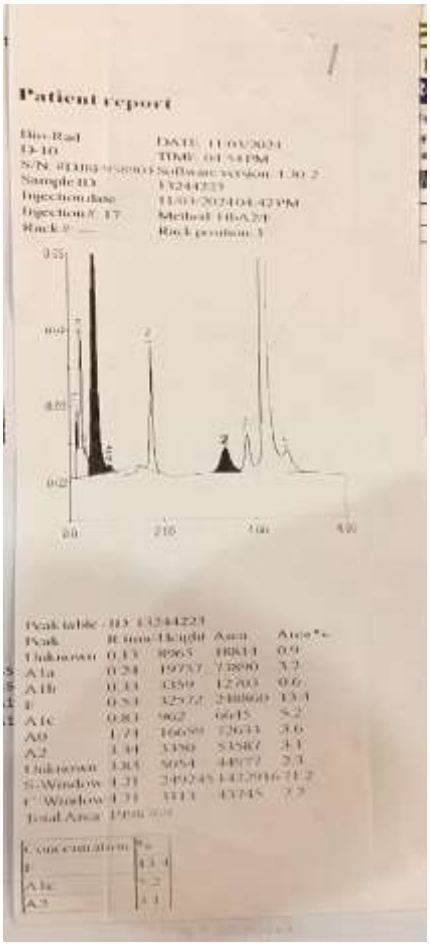
The majority of patients (150/200) exhibited a normocytic normochromic blood picture, with a mean MCV of 92.3 fL and MCH of 27.1 pg, reflecting normal red cell size and hemoglobin content. In contrast, 48 patients showed a microcytic hypochromic pattern characterized by significantly lower mean MCV (72.8 fL) and MCH (23.2 pg), typical of thalassemia and other microcytic anaemias. Only 2 cases demonstrated macrocytic anemia with elevated MCV (107 fL) and MCH (31.5 pg), a less

common finding in this cohort. This distribution underscores the predominance of normocytic and microcytic patterns in paediatric hemoglobinopathies.

Table 5: Geographic Distribution of Hemoglobinopathies in Vindhya Region.

Location	HBS Homozygous (Sickle cell disease)	Thalassemia Major	HBS Heterozygous (Sickle cell trait)	Double Heterozygous and Beta Thalassemia	Other	Total
Shahdol	08	06	07	03	01	25
Sidhi	06	04	04	03	03	20
Umaria	07	06	04	02	01	20
Rewa	08	04	01	01	01	15
Satna	04	04	01	01	00	10
Other	02	02	01	01	00	06
Total	35	26	18	11	06	96

The geographical distribution of hemoglobinopathies among the paediatric population reveals significant regional variations. Shahdol reported the highest number of cases (25 out of 96), with HbS homozygous (Sickle Cell Disease) being the most prevalent (8 cases), followed closely by HbS heterozygous (7 cases) and Thalassemia major (6 cases). Sidhi and Umaria both recorded 20 cases each, also dominated by HbS homozygous and Thalassemia major cases, suggesting a notable burden in these districts. Rewa accounted for 15 cases, while Satna reported the lowest among the major districts (10 cases), with a relatively even spread across the types. A small number of cases (6) came from other regions.



(HPLC – Sickle Cell Disease)

Discussion

The present study "**Hemoglobinopathies in Children of tribal region of Madhya Pradesh: Correlation of Hematological Indices and HPLC Findings** " was carried out in 200 patients.

Age and Gender:

In this study of 200 paediatric patients, the highest prevalence of hemoglobinopathies was in the 6–8 years age group (32%), followed by 9–10 years (25%), reflecting delayed diagnosis likely due to lack of universal new-born screening. Only 6% were diagnosed before 2 years of age, similar to findings by Rathod et al. (2012)[7]. Gender distribution was nearly equal (49% males, 51% females), consistent with autosomal recessive Inheritance.

Hematological Indices:

Mean hemoglobin was 8.9 ± 2.1 g/dL with microcytic hypochromic anemia (MCV 72.2 ± 8.7 fL, MCH 22.1 ± 3.5 pg) and elevated RDW (18.3 ± 3.2%), aligning with Ghosh et al. (2017)[8]. Reticulocyte count (5.33 ± 1.94%) and nucleated RBCs (7.3 ± 5.35) indicated compensatory erythropoiesis, especially in sickle cell disease.

Symptoms:

Weakness (55%) and pallor (41%) were most common, with icterus (16%) and splenomegaly/hepatomegaly (8%) less frequent.

Types of Hemoglobinopathies:

Sickle cell trait (20%) and sickle cell disease (14.5%) were predominant, followed by beta-thalassemia trait (6.5%) and beta-thalassemia major (2%). Double heterozygous sickle cell & beta-thalassemia cases (3%) highlight the need for comprehensive HPLC screening.

Haematological Indices by Hemoglobinopathy:

Sickle cell disease showed normocytic normochromic anemia (MCV 92.1 fL), while beta-thalassemia presented with microcytic hypochromic features (MCV 65.7–73 fL). Double heterozygous cases exhibited intermediate values.

Reticulocyte and NRBC Counts:

Elevated reticulocytes and nRBCs were noted in β-thalassemia major and sickle cell disease, while trait carriers showed lower counts, reflecting disease severity and marrow stress.

Geographical Distribution:

Highest prevalence was observed in tribal-dominant districts Shahdol, Sidhi, and Umaria, underscoring the need for focused screening and genetic counselling in these high-risk regions.

MCV and MCH Patterns:

Normocytic normochromic anemia predominated (75%), with microcytic hypochromic anemia in 24% cases. Macrocytic anemia was rare.

Conclusion

This study highlights the epidemiology and haematological features of paediatric hemoglobinopathies, emphasizing early detection in regions without new born screening. Sickle cell trait/disease and beta-thalassemia were most common. Key indices like MCV, MCH, and RDW aided diagnosis, supporting targeted screening and combined use of haematology and HPLC for accurate identification.

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