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Original Research Article

Pattern Analysis of Hb Variants by HPLC & its Clinico-Haematological Correlation in The Tertiary Health Care Centre of Northern Odisha, India

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Abstract:

Thalassemia and hemoglobinopathies are major health problems associated with significant morbidity and mortality in the Indian population. Hemoglobinopathies are inherited in an autosomal recessive manner and affect globin chains. Thalassemia is characterised by the absence or reduced synthesis of one or more globin chains. Sickle cell disease is the most common hemoglobinopathy, accounting for 7% of the world's population. Different Hb variants are relatively common in India compared to global data. Analysis of Hb variants by the HPLC method can help estimate the magnitude of the disorder and assist in early diagnosis and prevention through genetic counselling. This study aims to detect Hb variants using the HPLC technique and to determine the prevalence of hemoglobinopathies as well as their correlation with clinicohematological parameters. This study was conducted over the last two years in our tertiary care centre. The HPLC findings of Hb fractions were correlated with haematological parameters and clinical profiles. The results revealed that out of a total of 1,394 cases in the study, 326 had abnormal Hb variants. The most common Hb variant was β-thalassemia trait, accounting for 153 cases, followed by sickle cell trait with 71 cases, and 11 cases of β-thalassemia major. Other hemoglobinopathy cases included 20 cases of HbE trait, 21 cases of HbE homozygous, 20 cases of homozygous sickle cell disease, 2 cases of hereditary persistent fetal haemoglobin (HPFH), and 1 case of Hb Lepore. There were 27 cases of compound heterozygotes: 22 of which were for sickle cell/β-thalassemia, 4 for HbE/β-thalassemia, and 1 case of HbS/D trait.

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Introduction

Hemoglobinopathies, an inherited disease, cause a wide spectrum of clinical presentation ranging from mild anaemia to a life-threatening condition requiring blood transfusion for survival. Sickle cell disease represents one of the most prevalent haemoglobin disorders worldwide, accounting for 7% of the world population [1] and remains a major public health concern in several regions of India. HPLC (High-performance liquid chromatography has emerged as a dependable diagnostic modality for accurate diagnosis of hemoglobinopathies and thalassemia [2] due to its ability to separate multiple haemoglobin fractions with high precision and reproducibility. In the Indian subcontinent, the spectrum and frequency of different haemoglobin variants show considerable regional variation, underscoring the need for area-specific epidemiological data as compared to world data [3]. Systematic analysis of haemoglobin variants by using HPLC can therefore aid in assessing the disease burden as well as help in early diagnosis and prevention by targeted screening and genetic counselling [4].

Aims & Objectives: This study was conducted over the last two years to detect Hb variants from the HPLC technique and to determine the prevalence of hemoglobinopathies as well as

thalassemia, and correlate with clinico-haematological parameters.

Material & Methods

The study was carried out from July 2023 to June 2025. All cases undergoing HPLC examination were included in the study. Demographic profile, clinical presentation, and family history were collected where available. Complete Blood Count (CBC) parameters, Peripheral Blood Smear (PBS) findings were collected in each case using a proforma, which was then inserted into the master chart in an Excel sheet. These findings were correlated with the HPLC findings of Hb fractions and the haematological diagnosis. Supplementary investigations such as serum iron profiles & serum ferritin levels, and sickling tests were performed whenever necessary.

Statistical Analysis was done using SPSS software and Microsoft Excel. Data were expressed as mean and SD (standard deviation).

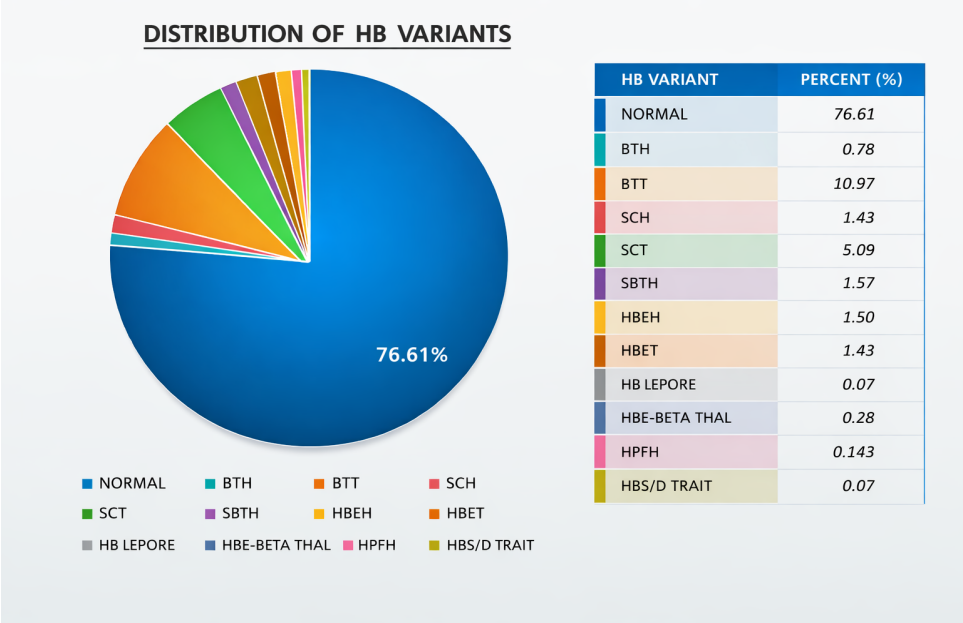
Inclusion Criteria

1. All cases of anaemia (<10 gm%) attending Paediatrics, Obstetrics & Gynaecology, and Medicine Outpatient Departments.
2. Above the age of 6 months.
3. Cases with family history of hemoglobinopathies & thalassemia.

Exclusion Criteria

1. H/O blood transfusion in the last 3 months.
2. Cases of other haemolytic anaemias & anaemias with other known causes.

Out of a total of 1,394 cases included in the study, 326 (23.38%) had abnormal Hb variants. Figure 1 shows the distribution of cases. The most common Hb variant was β-thalassemia trait, accounting for 153 cases (10.9%) of the total, followed by sickle cell trait with 71 cases (5.09%). The remaining cases included 11 cases of β-thalassemia major (0.78%), 20 cases of HbE trait, 21 cases of HbE homozygous, and 20 cases of homozygous sickle cell disease (1.43%). There were also 2 cases of hereditary persistent fetal haemoglobin (HPFH) and 1 case of Hb Lepore. Twenty-seven cases were compound heterozygotes: 22 of which were for sickle cell/β-thalassemia (1.57%), 4 for HbE/β-thalassemia (0.28%), and 1 case of HbS/D trait (0.07%). HbF was raised in 78 cases. The mean ± SD values of haematological parameters in each group are given in Table 4. The majority of cases revealed low MCV, low MCH, and microcytic hypochromic anaemia on peripheral blood smear. Most cases of β-thalassemia major were seen within 10 years of age, and the majority were female children. β-thalassemia trait was most common in the 20 to 40 year age group and more frequent in females (Table 1). Seventy-seven per cent of total cases presented with weakness and pallor, and 25 cases (2.4%)—including 11 cases of β-thalassemia major and 14 cases of sickle cell/β-thalassemia—had splenomegaly (Table 2). Various haemoglobin fractions obtained by HPLC, leading to presumptive diagnosis in each case, are shown in Table 3. The sickling test was positive in 85.56% of cases with homozygous sickle cell disease and in 52.17% of sickle cell trait cases (Table 6). Family counselling was achieved in most cases of β-thalassemia major and homozygous sickle cell disease (Table 7).



HB VARIANT	PERCENT (%)
NORMAL	76.61
BTH	0.78
BTT	10.97
SCH	1.43
SCT	5.09
SBTH	1.57
HBEH	1.50
HBET	1.43
HB LEPORE	0.07
HBE-BETA THAL	0.28
HPFH	0.143
HBS/D TRAIT	0.07

Figure 1: Distribution of HB variants

Table 1: Age Wise Distribution of Hb Variants

Age / Hb Variant	0–1 YRS (35 cases)	>1–18 YRS (107 cases)	>18–40 YRS (125 cases)	>40–60 YRS (19 cases)	>60 YRS (3 cases)	TOTAL (289)
Normal Hb	17	66	75	5	0	163
BTH	1	4	1	1	1	8
BTT	6	16	9	10	2	43
SCH	4	1	4	0	0	9
SCT	4	15	24	3	0	46
SBTH	0	1	1	0	0	2
HBEH	1	0	7	0	0	8
HBET	1	3	4	0	0	8
HB LEPORE	1	0	0	0	0	1
HBE-BETA THAL	0	1	0	0	0	1

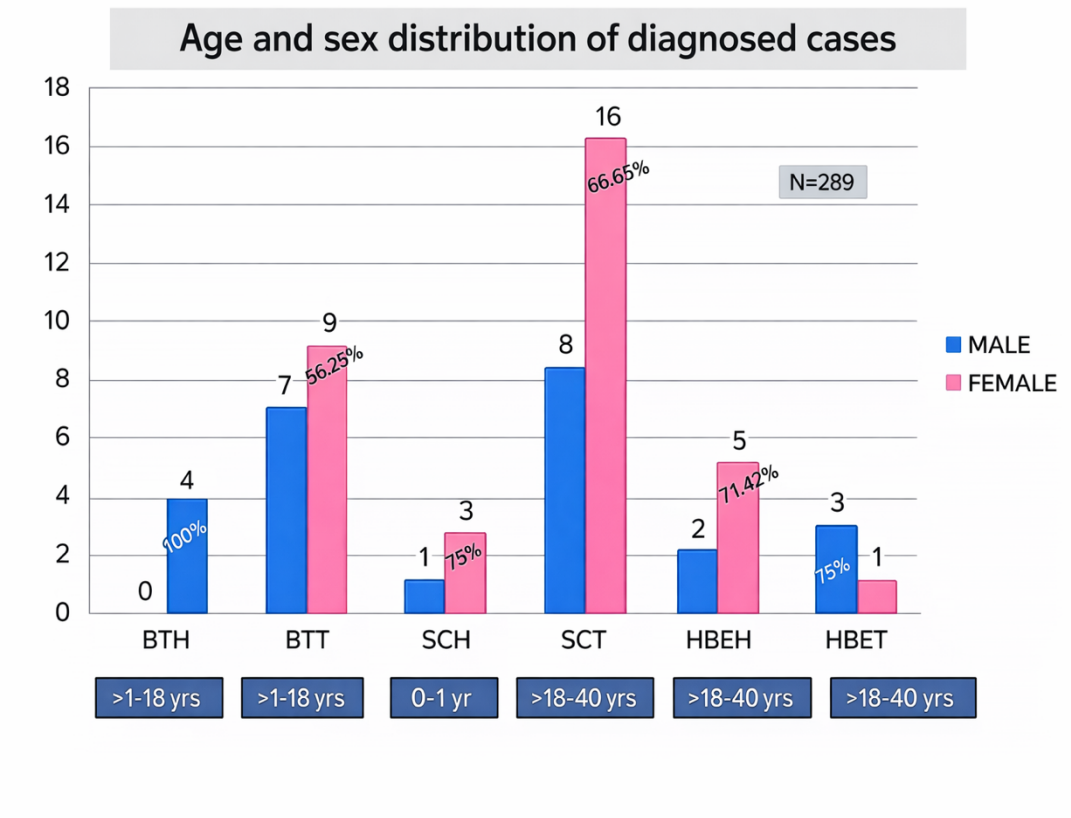


Figure 2: Age and sex distribution of diagnosed cases

Table 2: Clinical Findings

Clinical Findings	Number of Cases (N)	Percentage of Cases (%)
Weakness	225	77.85%
Fatigue	205	70.93%
Dyspnea	110	38.06%
Bone Pain	6	2.07%
Jaundice	3	1.03%
Spleenomegaly	7	2.40%

Table 3: Hemoglobin fraction with HPLC findings

Diagnosis	Hb A0 (mean ± SD)	Hb A2 / Hb E (mean ± SD)	HbF (mean ± SD)	Hb S (mean ± SD)	HbD
NORMAL HB	79.80 ± 11.55	2.23 ± 1.90	1.69 ± 3.33	nil	nil
BTH	13.13 ± 7.33	2.78 ± 1.87	80.75 ± 6.96	Nil	Nil
BTT	70.90 ± 14.26	6.58 ± 2.68	4.03 ± 3.81	Nil	nil
SCH	21.03 ± 12.6	2.5 ± 0.827	13.92 ± 2.46	64.5 ± 11.35	nil
STT	61.53 ± 6.88	2.45 ± 0.64	1.61 ± 1.26	31.16 ± 6.61	nil
S-beta thal	17.3 ± 20.28	6 ± 16.83	26 ± 12.68	36.7 ± 18.42	nil
HBEH	35.75 ± 15.50	86.21 ± 20.50	7.95 ± 0.30	nil	nil
HBET	64.45 ± 4.34	35.225 ± 6.84	2.26 ± 0.52	nil	nil
HB LEPORE	82.3	22.4	2.2	nil	nil
HbE-BETA THAL	11.3	56.8 ± 3.84	20.6 ± 6.82	NIL	nil
HPFH	71.3 ± 4.67	2.85 ± 0.35	25.2 ± 0.14	nil	nil
HBS/D TRAIT	69.6	2.7	0.6	5.9	8

Table 4: Distribution of CBC Parameters with Hb Variants

Diagnosis	Total Cases (N)	% Cases	Age (Mean ± SD)	RBC (Mean ± SD)	Hb (Mean ± SD)	MCV (Mean ± SD)	MCH (Mean ± SD)	RDW (Mean ± SD)
Normal Hb	1068	76.61	17.73 ± —	4.30 ± 2.01	9.60 ± 6.63	67.66 ± 13.21	22.41 ± 5.31	18.29 ± 4.81
BTH	11	0.78	19 ± 23.02	3.59 ± 1.24	7.125 ± 1.97	59.53 ± 6.48	21.8 ± 4.17	20.8 ± 9.11
BTT	153	10.97	24.85 ± 20.13	4.1 ± 1.02	8.7 ± 2.09	67.3 ± 6.34	20.97 ± 2.80	17.79 ± 4.98
SCH	20	1.43	12.55 ± 11.57	3.16 ± 0.37	8.68 ± 1.61	81.82 ± 12.44	25.51 ± 2.62	20.31 ± 3.28
BCT	71	5.09	23.95 ± 13.83	5.07 ± 4.48	9.63 ± 3.57	70.92 ± 13.36	23.12 ± 4.62	17.38 ± 4.05
Sickle-BT	22	1.57	14 ± 6.63	2.8 ± 1.01	6.75 ± 2.46	80.7 ± 12.05	29.15 ± 4.80	24.75 ± 5.69
HBEH	21	1.5	19.75 ± 7.88	3.77 ± 0.93	8.45 ± 1.65	63.95 ± 8.64	22.45 ± 2.01	21.48 ± 4.45
HBET	20	1.43	14.5 ± 11.17	3.01 ± 1.13	7 ± 1.45	68.51 ± 12.14	21.67 ± 4.02	19.325 ± 3.87
HB Lepore	1	0.07	1	4.5	9.1	64.9	26.3	13.7
HbS-Beta Thal	4	0.28	1	3.84	8.1	71.1	16.7	18.6
HPFH	2	0.143	28.5	3.62	8.35	73	25.2	15

Table 5: Distribution of Haematological Diagnosis According to Red Cell Morphology

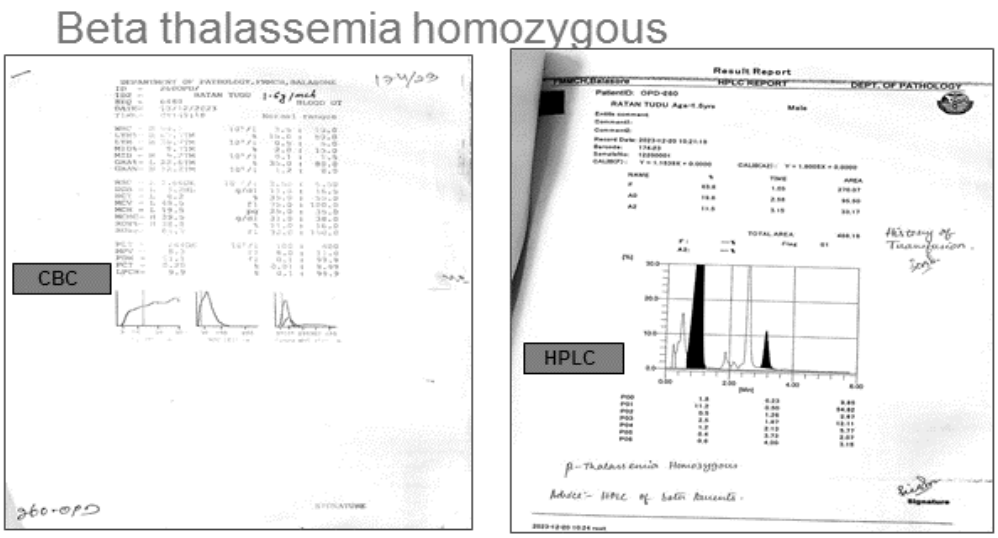
Hematological Diagnosis	Normocytic Normochromic (Cases)	Normocytic Normochromic (%)	Microcytic Hypochromic (Cases)	Microcytic Hypochromic (%)
Normal Hb	28 cases	17.17%	135 cases	82.82%
BTH	-	-	8 cases	100%
BTT	-	-	43 cases	100%
SCH	6 cases	66.66%	3 cases	33.33%
SCT	12 cases	26.08%	34 cases	73.91%
Sickle-Beta Thal	2 cases	100%	-	-
HBEH	-	-	8 cases	100%
HBET	2 cases	25%	6 cases	75%
Hb Lepore	-	-	1 case	100%
HBE-Beta Thal	-	-	1 case	100%

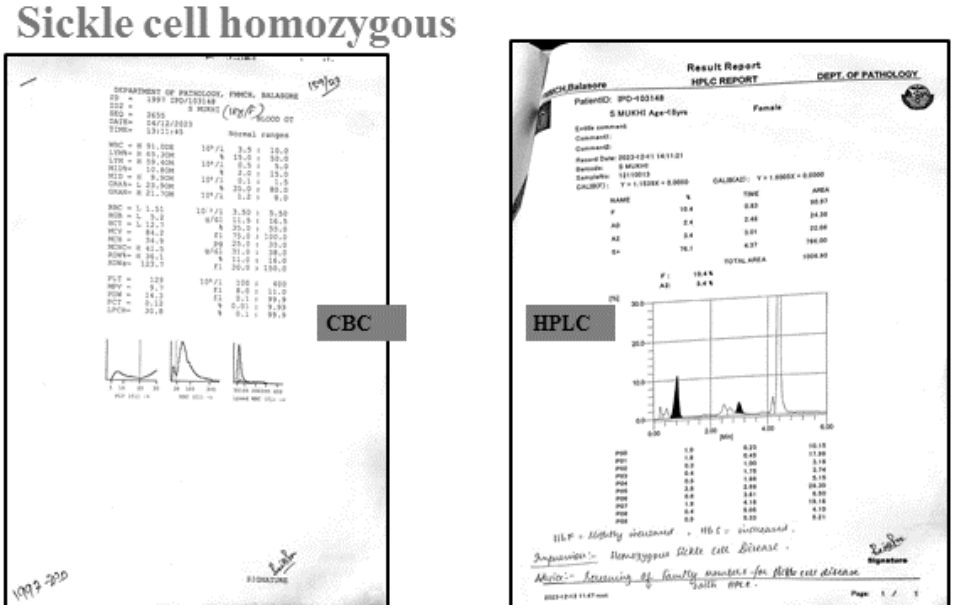
Table 6: Sickling Test

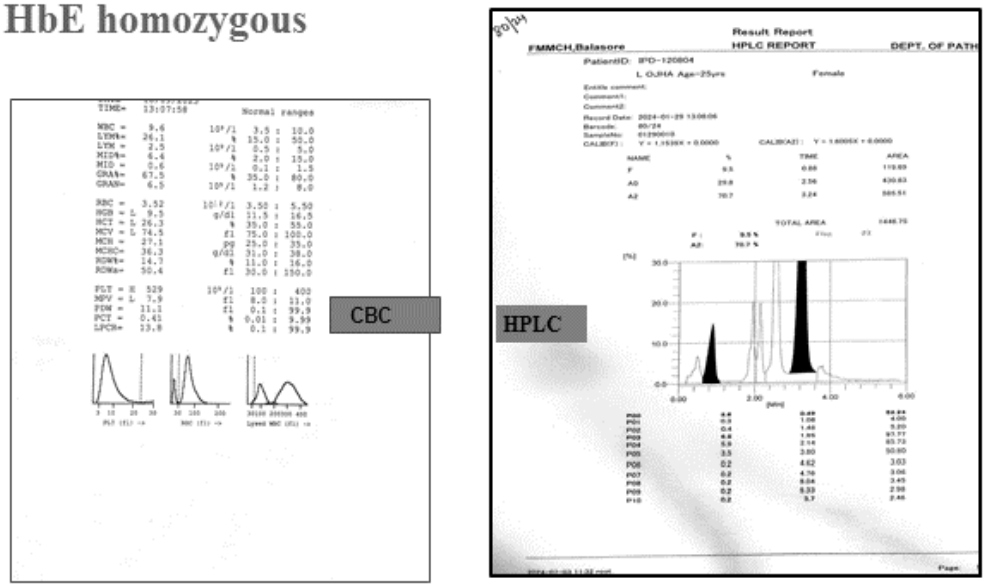
Sickling Test	Positive (Cases)	Positive (%)	Negative (Cases)	Negative (%)	Not Done (Cases)
Sickle Cell Homozygous (SCH) *(n = 9)*	5	55.56%	2	22.22%	2
Sickle Cell Trait (SCT) *(n = 46)*	24	52.17%	10	21.73%	12

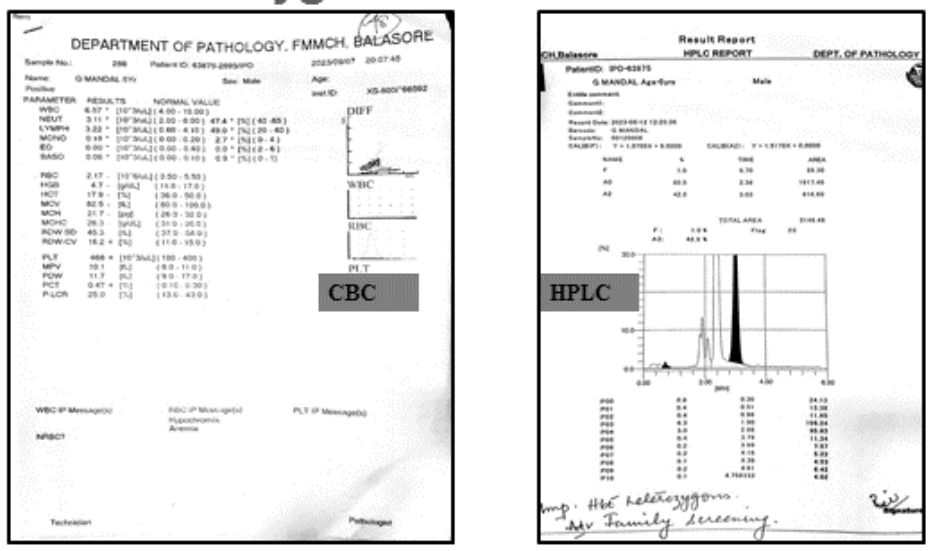
Table 7: Family Counselling

Family Counselling	Done	Family Details	Total Cases
Beta Thalassemia Trait (BTT)	2	Father – Normal, Mother – BTT	43
		Father – Normal, Mother – BTT	
Beta Thalassemia Homozygous (BTH)	1	Father – BTT, Mother – BTH	5
Sickle Cell Homozygous (SCH)	2	Sister – SCH, Brother – SCT	9
		Father – SCT, Mother – SCT	
Sickle Cell Trait (SCT)	3	Father – SCT, Mother – Normal	46
		Father – SCT, Mother – Normal	
		2 Siblings – SCT	









Discussion

Haemoglobinopathies and thalassaemia are the most common genetic disorders in the world. Thalassaemia is characterised by quantitative abnormalities in one or more of the globin chains. Specific mutations lead to the production of haemoglobin variants with low structural variations, like HbE and Hb Lepore, and hyper-unstable haemoglobin variants. Around 7 per cent of the population worldwide are carriers of these abnormalities, and more than 3,00,000 severely affected babies are born every year. Worldwide, the Hb disorders are responsible for 3.4% mortality in children below 5 years of age. Inherited disorders of abnormal haemoglobin are extremely common in the Indian population, ranging from structurally normal haemoglobins to severe transfusion-dependent haemoglobinopathies. HPLC is a rapid, automated, and accurate technique for the identification and quantification of haemoglobin fractions, enabling the detection of both common and rare variants [12]. Recognition of this rare Hb variant can contribute to improved clinical diagnosis and management. Also, it facilitates prenatal diagnosis and genetic counselling for these patients.

In our study conducted at the tertiary care centre F.M. MCH Balasore, Odisha, haemoglobin disorders were high at 23.38% compared to other significant population-based investigations by Mondal et al. [5], Bhalodia et al. [6], which reported prevalences of 12.17% and 8.6%, respectively, but at par with Manger et al. [7] at 22.24%. The most common Hb variant was β-

thalassemia trait in our study, accounting for 153 cases (10.9%) of total cases, similar to Manger et al. (11.55%) [7], higher than Mondal et al. (4.60%) [5], and Bhalodia et al. (5.20%) [6]. Figure 1 shows the distribution of cases. Out of 153 patients with the β-thalassemia trait, 16 cases (10.45%) were found to be iron-deficient with a recorded mean serum ferritin of 12.8 ng/ml. HbA2 levels in these cases were found to be significantly lower than cases with normal serum ferritin levels. Iron deficiency can lead to reduced levels of HbA2, potentially masking the presence of β thalassemia trait. In contrast, insufficient levels of vitamin B12 or folate may elevate HbA2 values, leading to a misdiagnosis of thalassemia traits in patients. The next majority case was sickle cell trait, 71 cases (5.09%), high in comparison to the study by Manger et al. [7], which reported 2.95%. Homozygous sickle cell disease in our study was 20 cases (1.43%), higher than Manger et al. [7]'s study, which accounts for about 0.76%. β-thalassemia major was detected in 11 cases (0.78%) in this study, higher than in research conducted by Khera R, Singh T et al [4] (0.4%) and lower than in the study by Manger et al. [7]. 1.17%. Other hemoglobinopathy cases included 20 cases of HbE trait (1.43%) and 21 cases of HbE homozygous (1.5%), approximately similar to Lakshmipriya et al. [8], with 14 cases of HbE trait and 22 cases of HbE homozygous out of 264 hemoglobinopathies. 2 cases of hereditary persistent fetal haemoglobin (HPFH), 0.143% of cases in this study, were seen in a 36-year-old and a 40-year-old male having HbF 25.2 ± 0.14 without any symptoms discovered during family screening of a child with the beta thalassaemia trait. Sirisa Patel & S Dehuri et al. found a similar incidence: 5 cases in one family in Balangir, Odisha, with HbF 26.1 ± 3.23%. (Journal of Clinical and Diagnostic Research OD09-10, 2015) [11]

In our study, 27 cases were compound heterozygotes, 22 of which were for sickle cell-β-thalassemia (1.57%), 4 for HbE/β-thalassaemia (0.28%), and 1 case (0.07%) for HbS/D trait. Our findings align with Ravi Shankar et al. [9] study conducted on hemoglobinopathies, who reported a comparable frequency of HbS/D (2 out of 120 cases).

β-thalassemia trait in our study showed RDW-CV of 17.79 ± 4.98, HbA2 of 6.58 ± 2.68, and MCV of 67.3 ± 6.34. Sickle cell trait and sickle cell beta thalassaemia in our study were HbF-1.61 ± 1.26, 26 ± 12.68, HbS-31.16 ± 6.61, and 36.7 ± 18.42, respectively. HbE trait & HbE-thal showed A2-35.225 ± 6.84 & 56.8 ± 3.84, and HbF-2.26 ± 0.52 & 20.6 ± 6.82, respectively. The CBC & HPLC metrics of the present study align closely with observations reported by Khera R, Singh T et al. [4] and Phalak et al. [10].

Correlation of HPLC results with CBC parameters & family studies can aid in the diagnosis of the majority of hemoglobinopathies and thalassaemia syndrome. β-thalassemia trait is very common in this part of the Indian subcontinent, and this study can help in early detection with timely intervention and facilitate family-based screening and genetic counselling.

Conclusion

- HPLC is a sensitive, specific, accurate & valuable alternative to electrophoresis in detecting & quantifying different Hb fractions.
- Retention time and percentage of variant Hb can provide important clues in differentiating various types of Hb eluting in the same window.
- Current guidelines require that an abnormal variant Hb should be confirmed by another independent technique.

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