

Association of Consanguinity and Family History with Compound Heterozygous Hemoglobinopathies: A Hospital-Based Study of Sickle- β -Thalassemia and HbE- β -Thalassemia in Eastern India

Monika Bharti¹, Mayank Raj², Ekta Kumari³

¹Senior Resident, Department of Pathology, Sheikh Bhikhari Medical College, Hazaribagh, Jharkhand, India

²Assistant Professor, Department of Community Medicine, Birsa Institute of Medical Sciences and Research, Khunti, Jharkhand, India

³Senior Resident, Department of Pathology, Sheikh Bhikhari Medical College, Hazaribagh, Jharkhand, India

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Corresponding Author: Ekta Kumari

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Abstract:

Background: Hemoglobinopathies are inherited disorders caused by structural abnormalities or defective synthesis of globin chains and constitute an important public-health problem in India. Compound heterozygous conditions, particularly sickle- β -thalassemia (S β -thalassemia) and HbE- β -thalassemia, demonstrate variable clinical and hematological manifestations and may pose diagnostic challenges. Consanguinity and family history are important epidemiological factors because inheritance of two abnormal β -globin alleles depends on the carrier status of both parents. High-performance liquid chromatography (HPLC), supported by hematological assessment and family studies, is useful for identifying these disorders.

Objective: To determine the occurrence of S β -thalassemia and HbE- β -thalassemia and evaluate the association of consanguinity and family history with compound heterozygous hemoglobinopathies in a hospital-based population from eastern India.

Methods: A hospital-based cross-sectional descriptive study was conducted among 207 patients evaluated for suspected hemoglobinopathy in the Department of Pathology at Rajendra Institute of Medical Sciences, Ranchi, Jharkhand. Patients with anemia, hepatosplenomegaly, suspected hemolytic anemia, positive sickling test or relevant family history were included. Demographic and clinical details, consanguinity, family history, complete blood count, peripheral smear, sickling test and HPLC findings were recorded. Hemoglobin fractions were analysed using cation-exchange HPLC. Categorical variables were compared using Fisher's exact test, and $p < 0.05$ was considered statistically significant.

Results: Among 207 samples, 24 (12.0%) demonstrated compound/double heterozygous hemoglobinopathies, comprising 20 (83.3%) S β -thalassemia and 4 (16.7%) HbE- β -thalassemia cases. In the S β -thalassemia group, 11 (55%) were males and 9 (45%) females, while HbE- β -thalassemia showed equal sex distribution (2 males and 2 females; $p = 1.000$). Ten (50%) S β -thalassemia and three (75%) HbE- β -thalassemia patients were aged ≤ 18 years ($p = 0.596$). Consanguinity was documented in 8/24 (33.3%) cases, including 6/20 (30%) S β -thalassemia and 2/4 (50%) HbE- β -thalassemia cases ($p = 0.578$). S β -thalassemia demonstrated mean hemoglobin of 8.16 g/dL, MCV 73.89 fL, MCH 25.4 pg and MCHC 31.34 g/dL. Corresponding values for HbE- β -thalassemia were 6.98 g/dL, 79.7 fL, 24.3 pg and 29.0 g/dL. Family studies were performed in seven S β -thalassemia cases and confirmed the diagnosis. Family confirmation could not be completed in the four HbE- β -thalassemia cases because of logistical limitations.

Conclusion: Compound heterozygous hemoglobinopathies constituted an important proportion of hemoglobinopathy cases in this hospital-based population. One-third of affected individuals reported consanguinity, emphasizing the relevance of detailed family and marital history. HPLC combined with family studies can improve recognition of compound heterozygous states and facilitate genetic counselling, carrier screening and preventive strategies in high-risk populations.

Keywords: S β -thalassemia; HbE- β -thalassemia; compound heterozygosity; consanguinity; family history; HPLC; hemoglobinopathy; Jharkhand; India.

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Introduction

A diverse range of hereditary conditions known as hemoglobinopathies are caused by structural defects or poor globin chain production. With significant geographical and ethnic heterogeneity, sickle haemoglobinopathies and β -thalassemia are significant public health issues in India. Disorders like sickle- β -thalassemia and HbE- β -thalassemia are caused by compound heterozygosity, which is the inheritance of two distinct aberrant β -globin alleles [1]. The underlying β -thalassemia mutation, haemoglobin composition, and modifying genetic variables all affect clinical severity. Because it enables the quick detection and measurement of aberrant haemoglobin fractions, HPLC is a crucial diagnostic technique. Current research in India highlights the need of HPLC-based screening and continues to show significant geographical variation in hemoglobinopathies. The likelihood that people with harmful recessive alleles will have impacted offspring is increased by consanguinity. In order to understand complicated HPLC results and confirm compound heterozygous situations, family history and parental studies are crucial [2, 3].

Sickle-cell and thalassaemia are prevalent in people in Eastern India, particularly Jharkhand and surrounding states. The goal of the current study was to determine how common S β -thalassemia and HbE- β -thalassemia are in a hospital-based population, as well as how these illnesses relate to consanguinity and family history [4].

Methods

Study Design and Setting: This was a hospital-based cross-sectional descriptive study conducted in the Department of Pathology at Rajendra Institute of Medical Sciences, Ranchi, Jharkhand.

A total of 207 samples were included for evaluation of suspected hemoglobinopathies.

Study Population: Patients attending outpatient and inpatient services with clinical or hematological suspicion of hemoglobinopathy were considered for evaluation. The study population included individuals presenting with anemia, hepatosplenomegaly, suspected hemolytic anemia, positive sickling test or a relevant family history of hemoglobinopathy.

Inclusion Criteria

Patients fulfilling one or more of the following criteria were included:

1. Anemia with clinical suspicion of hemoglobinopathy.
2. Positive sickling test.
3. Hepatosplenomegaly with suspected hemolytic anemia.

4. Clinical or hematological features suggestive of thalassemia.
5. Family members of patients with suspected or confirmed hemoglobinopathy.
6. Patients with available demographic, clinical and laboratory information.

Exclusion Criteria

Patients were excluded if:

1. They had received blood transfusion within the preceding 6 months.
2. They declined testing.
3. Adequate clinical or laboratory information was unavailable.
4. HPLC results were uninterpretable or inadequate for classification.

The source thesis similarly excluded recently transfused patients because transfused red cells can alter the observed hemoglobin fractions.

Clinical and Demographic Assessment: A structured proforma was used to record age, sex, religion/ethnic background, socioeconomic characteristics, clinical presentation, history of anemia, jaundice, hepatosplenomegaly, pain episodes, previous hospitalization, blood transfusion, consanguinity and family history. The original study proforma specifically included consanguineous marriage, family history, sibling history, clinical manifestations and HPLC findings.

Haematological Investigations: Venous blood was collected in EDTA-containing tubes. Complete blood count and red-cell indices, including hemoglobin, RBC count, MCV, MCH and MCHC, were evaluated. Peripheral blood smears were examined following Leishman staining.

The source study demonstrated a predominantly microcytic-hypochromic hematological profile in both compound heterozygous groups.

Sickling Test: Sickling screening was performed using the sodium metabisulfite method. A freshly prepared reducing solution was used to induce reduced oxygen tension, and the preparation was examined microscopically for characteristic sickling.

HPLC Analysis: Hemoglobin fractions were evaluated using cation-exchange high-performance liquid chromatography. The method permitted identification and quantification of HbA, HbA₂, HbF and abnormal fractions such as HbS and HbE. HPLC retention time and peak characteristics were interpreted in conjunction with CBC, peripheral smear and clinical information.

The source study used HPLC to classify cases based on HbS, HbF, HbA₂ and HbA₀ fractions and

emphasized that family studies may be necessary in difficult cases.

Family Study: Family evaluation was performed whenever feasible. Parents and/or other first-degree relatives were evaluated using CBC and HPLC to establish carrier status and support confirmation of the compound heterozygous diagnosis.

In the source dataset, family studies could be performed in 7 out of 20 S β -thalassemia cases, confirming the diagnosis. The remaining cases could not undergo complete family evaluation because of lack of awareness, logistical difficulties, financial constraints, parental reluctance and death of a parent.

Assessment of Consanguinity: Consanguinity was recorded based on reported history of marriage between biologically related individuals. The original study documented consanguineous

marriage in 6 S β -thalassemia and 2 HbE- β -thalassemia cases, giving an overall frequency of 8/24 (33.33%).

Statistical Analysis: Data were summarized as frequencies and percentages for categorical variables and mean values for continuous hematological variables. Fisher's exact test was applied to comparisons involving small cell counts. A p-value <0.05 was considered statistically significant.

Note: The uploaded source does not provide sufficient variance estimates for hemoglobin, MCV, MCH or MCHC to calculate valid comparative p-values for those continuous parameters. Therefore, p-values for these variables have not been fabricated.

Results

Table 1: Age and Sex Distribution Amongst Groups

Group A Sickle Beta Thalassemia (N=20)

Age (Years)	Male	Female	Total	%
0 To 18	5	5	10	50
19 To 40	4	5	9	45
> 40	1	-	1	5

Group B Hbe Beta Thalassemia (N= 4)

Age (Years)	Male	Female	Total	%
0 To 18	1	2	3	75%
19 To 40	1	-	1	25%
> 40	-	-	-	-

Table 2: Caste Based Distribution of Cases

Caste	Group A (N=20)	Group B (N=4)
General	4	1
Muslims	5	2
Scheduled Castes	2	1
Scheduled Tribes	6	-
Other Castes	3	-

■ GEN ■ MUS ■ ST ■ SC ■ OC

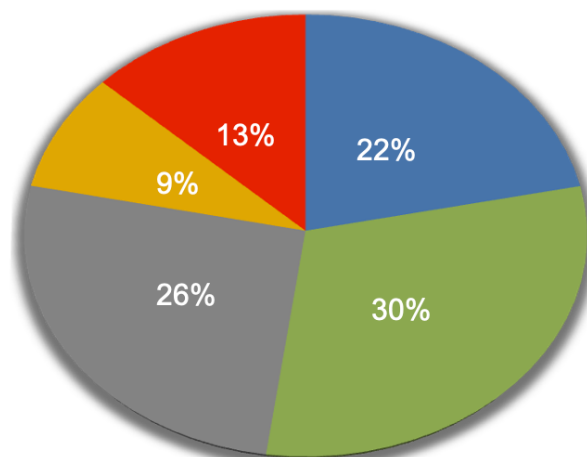
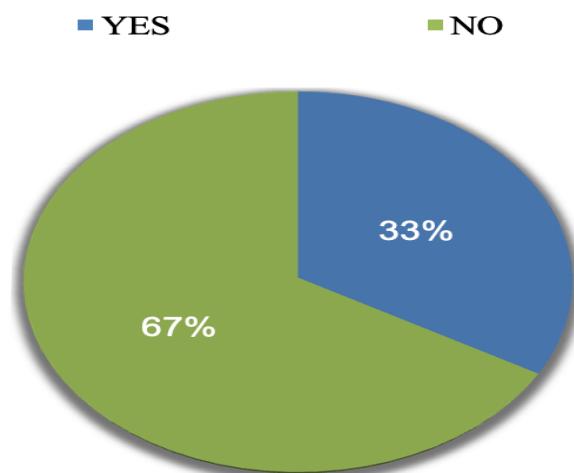


Chart 1: Caste Distribution of Cases:

Table 3: History of Consanguinity

Group	Group A SB Thal	Group B EB Thal	%
H/O Consanguinous Marriage	6/24	2/24	33.33

**Chart 2: Co-Relation With Consanguinity****Table 4: Mean Hematological Parameters In All**

Group	Group A SB Thal	Group B EB Thal
HB (gm%)	8.16	6.98
MCV (fl)	73.89	79.7
MCH (pg)	25.4	24.3
MCHC (gm%)	31.34	29

Both the groups show value of haemoglobin to lesser than normal level. Values of MCV remains at the lower range for both the groups. Values of MCH remains lower in both the groups. MCHC also lies in lower ranges as compared to normal.

Table 5: Average Hemoglobin Fraction in Groups

Group	Group A SB Thal	Group B EB Thal
HbF (%)	25.9	17.75
HbA ₂ (%)	4.08	67.93
HbS (%)	65.11	-

Value of HbF remains high in both the groups in group A as well as group B. Value of HbA₂ is higher in group B. HbS remains moderately high in sickle beta thalassemia.

Table 6: Socio-Economic Correlation

Socio - Economic Class	Group A	Group B	Total
Class I	-	-	-
Class II	-	-	-
Class III	9	3	12
Class IV	10	1	11
Class V	1	-	1
Class VI	-	-	-

Socio economic categorisation is done using Kuppuswamy socio economic status scale 2022. The categorisation is done based on monthly income of the family, Educational qualification of head of family, and Occupation of head of family.

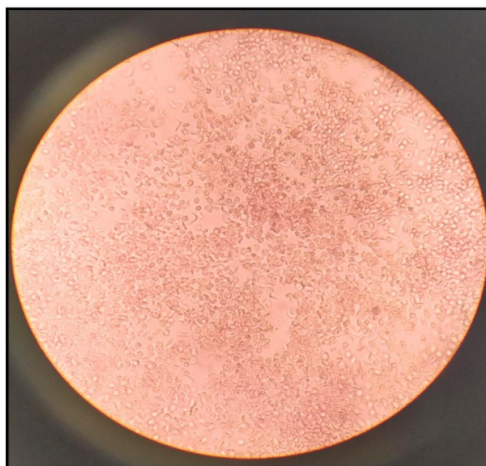


Figure 1: Positive sickling test showing Sickled RBC

Discussion

The present hospital-based cross-sectional study evaluated 207 individuals with suspected hemoglobinopathy and identified 24 cases of compound/double heterozygous hemoglobinopathy. Twenty patients had S β -thalassemia, which was the most common disease, whereas four patients had HbE- β -thalassemia. As a result, 83.3% of compound heterozygous patients had S β -thalassemia, whereas 16.7% had HbE- β -thalassemia. In eastern India, where HbS and β -thalassemia alleles coexist in multiple populations, this prevalence is clinically significant. The distribution of haemoglobin variations varies significantly geographically, according to recent Indian data, which supports region-specific screening strategies. With significant geographical variation, a recent systematic review of 99 Indian papers found pooled prevalence estimates of 2% for sickle cell anaemia, 5% for sickle cell trait, 6% for β -thalassemia, and 7% for β -thalassemia trait [5].

The current study's S β -thalassemia predominance is also in line with a recent cross-sectional survey conducted in eastern India in 2026, which found that 60 out of 70 double heterozygous cases (85.7%) had S β -thalassemia and 10 (14.3%) had HbE- β -thalassemia. The majority of the patients in that study had moderate-to-severe microcytic hypochromic anaemia, and pallor and splenomegaly were noted as common clinical symptoms. The finding that S β -thalassemia is a significant compound hemoglobinopathy in this area is reinforced by the similar distribution. In the current study, HbE- β -thalassemia exhibited an equal sex distribution, whereas S β -thalassemia afflicted males and females in comparatively similar proportions. At $p=1.000$, the difference was not statistically significant [6].

This result is anticipated as β -globinopathies do not exhibit a primary sex-linked tendency due to their autosomal recessive inheritance pattern. Therefore, it is not appropriate to interpret the comparatively

higher incidence of S β -thalassemia in men as proof of sex-specific vulnerability. The age distribution revealed that three-fourths of patients with HbE- β -thalassemia and half of those with S β -thalassemia were under the age of eighteen. At $p=0.596$, the difference was not statistically significant. Because compound heterozygous situations can result in chronic anaemia, jaundice, splenomegaly, and other problems needing long-term surveillance, early presentation is clinically significant. According to the source study, HbE- β -thalassemia was more generally identified in the second decade, while S β -thalassemia typically manifested in the late first decade [9, 10].

The haematological results showed that both groups had anaemia and mostly microcytic/hypochromic red-cell indices. The mean haemoglobin was 6.98 g/dL in HbE- β -thalassemia and 8.16 g/dL in S β -thalassemia, indicating that individuals with HbE- β -thalassemia had comparatively more anaemia. MCH and MCHC decreased in both groups as well. These findings align with current data that indicates HbE- β -thalassemia patients have more severe anaemia and anisocytosis than S β -thalassemia patients. In comparison to S β -thalassemia, HbE- β -thalassemia had much higher RDW and significantly lower haemoglobin, PCV, MCH, and MCHC in the 2026 Eastern Indian research [7].

Consanguinity was one of the main topics of this investigation. Eight out of 24 compound heterozygous patients (33.3%), comprising two HbE- β -thalassemia cases and six S β -thalassemia cases, reported consanguineous marriage. The overall frequency is epidemiologically relevant even if the difference between the two groups was not statistically significant ($p=0.578$). The likelihood that two people with pathogenic recessive alleles will have children who inherit defective alleles from both parents is increased when two people are consanguineous. Consanguinity was also noted as a significant element in the source material, with Muslim and Scheduled Tribal populations having

the highest prevalence. Because community-specific haemoglobin variant distribution, endogamy, and population structure can all affect the incidence of compound diseases, this observation is especially significant in eastern India [8].

According to a recent study from Bihar, 35 out of 2,329 people who were examined had double heterozygous HbE/ β -thalassemia, highlighting the ongoing prevalence of hemoglobinopathies in eastern India. Similar to this, research on Indian tribal tribes has shown that factors related to hemoglobinopathy screening include genetic clustering, consanguinity, and limited access to healthcare. In the current study, family studies offered crucial diagnostic corroboration. Seven of the twenty instances of β -thalassemia were able to undergo family studies, which validated the diagnosis. However, due to low knowledge, logistical and budgetary limitations, parental resistance, and parent death, a full family evaluation was not possible for the remaining patients. This illustrates a real-world difficulty in diagnosing hemoglobinopathy in hospitals. [9].

HPLC may strongly suggest a compound heterozygous state, but parental or sibling studies can provide valuable evidence regarding inheritance and help distinguish complex patterns. HPLC remains particularly useful because it simultaneously identifies and quantifies HbS, HbF, HbA₂ and HbA fractions. Recent evidence supports its role as an efficient first-line diagnostic approach. A multicentre Indian study involving 6,549 antenatal women reported that an HPLC-first strategy identified approximately 14% more carriers without increasing overall screening costs. Another recent study of 1,900 samples highlighted both the diagnostic value of HPLC and the challenges of interpreting abnormal chromatographic peaks in routine practice [10].

However, there are restrictions with HPLC. In β -thalassemia, it might not be able to distinguish β - from β^+ mutations with certainty, and in circumstances where diagnosis is challenging, molecular testing might be necessary. Additionally, the source study found that haemoglobin levels alone were insufficient to consistently discriminate between β^+S and β^+S . The use of haemoglobin fraction analysis in conjunction with molecular testing when appropriate is also emphasised in recent Indian recommendations. The consequences for clinical practice are significant. When a patient is found to be compound heterozygous, testing first-degree relatives and, if applicable, the reproductive partner should be considered. Severe hereditary haemoglobin abnormalities can be prevented with prenatal diagnosis, antenatal screening, genetic counselling, and carrier detection. The current Indian guidelines for sickle cell disease and

thalassaemia place a strong emphasis on precise diagnosis, systematic screening, and genetic counselling for prevention [11].

This study's single-centre design, very small number of compound heterozygous cases, and insufficient family investigations are its main drawbacks. The accurate classification of $S\beta^0$ and $S\beta^+$ diseases are also limited by the lack of molecular proof. Additionally, the study cohort may not accurately reflect the prevalence of these disorders in the broader population because it was hospital-based. Overall, the results highlight the need of routinely considering family history and consanguinity when evaluating suspected compound hemoglobinopathies. The most accurate diagnosis and effective prevention are likely to come from a diagnostic approach that combines CBC, peripheral smear, sickling testing, HPLC, and family studies, with molecular testing added as needed [12].

Conclusion

In eastern India, compound heterozygous hemoglobinopathies, including $S\beta$ -thalassemia and HbE- β -thalassemia, make up a significant portion of the hemoglobinopathy spectrum. $S\beta$ -thalassemia predominated among the 24 instances found in the current 207 sample hospital-based investigation. Anaemia and mostly microcytic/hypochromic red-cell indices were linked to both conditions, whereas HbE- β -thalassemia had a significantly lower mean haemoglobin content.

In 33.3% of compound heterozygous instances, consanguinity was found, highlighting the significance of a thorough marital and family history throughout the diagnostic process. The frequency found supports focused family and community screening in populations with a high burden of haemoglobin variations, even though the correlation between consanguinity and specific disease subtype was not statistically significant.

Family studies were useful for confirming $S\beta$ -thalassemia, but they were constrained by logistical, economical, and awareness-related issues. Although molecular testing may be necessary for definitive characterisation of β -thalassemia alleles, HPLC is still a useful and efficient diagnostic method. The incidence of severe hemoglobinopathies in Jharkhand and surrounding areas might be significantly decreased by bolstering carrier screening, cascade testing, genetic counselling, prenatal screening, and preventive interventions.

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