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Original Research

# Epidemiological Profile of Haemoglobinopathies in Different Districts of West Bengal: A Retrospective Study

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## Abstract

**Background:** Haemoglobinopathies are autosomal recessive inherited disorders affecting haemoglobin structure or production. Over 1,000 haemoglobin variants have been identified, with carriers often asymptomatic or exhibiting mild anaemia. When both parents are carriers, there is a 25% risk per pregnancy of having a child with a severe form of the disease. Our study aims to (i) describe the epidemiological profile of different haemoglobinopathies, (ii) evaluate the variety of haemoglobinopathies and carriers, and(iii) estimate the prevalence of haemoglobinopathies and carriers.

**Methodology:** This retrospective cross-sectional study included 5,000 cases obtained from multiple screening camps organized by Ma Sarada Charitable Dispensary & Pathology Centre. All available high-performance liquid chromatography (HPLC) reports with corresponding patient clinical histories and complete blood count results were reviewed. Patients with a history of blood transfusion were excluded from the study to prevent alteration of hemoglobin profiles. Data was extracted and analysed to determine the prevalence and types of haemoglobinopathies in the study population.

**Result:** Beta thalassemia carrier is the most common haemoglobinopathy (6.36%) detected in West Bengal, followed by HbE carrier. HbE disease, Hb E beta thalassemia, Hb S carrier, Hb S disease, and HPHF trait are the other haemoglobinopathies, also found in this study. Most of the patients are male (11.27%). Among the districts, Beta thalassemia carrier is mostly found in North 24 Parganas, and Hb E carrier is mostly found in South 24 Parganas. Other haemoglobinopathies are also mostly found in North 24 Parganas.

**Conclusion:** This study highlights a significant prevalence of beta thalassemia and other hemoglobinopathies in the screened population, underscoring the urgent need for widespread screening programs to identify asymptomatic carriers. Early detection through high-performance liquid chromatography (HPLC) can facilitate timely genetic counseling and intervention, thereby helping to prevent disease transmission. Although HPLC interpretation demands specialized training, it remains a reliable and practical screening tool when performed promptly after blood collection to minimize diagnostic errors.

**Keywords:** Haemoglobinopathy; Beta Thalassemia Trait; Hb E Carrier; HPLC.

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**Quick Response Code:**

## Introduction

Haemoglobinopathies, including thalassemia and sickle cell diseases, are one of the common inherited disorders with variable prevalence and genetic characteristics. These are autosomal recessive disorders characterized by either reduced synthesis of Hb polypeptide chains in red blood cells (thalassemia) or structural changes in Hb (sickle cell disease-SCD) [1]. Inherited haemoglobin disorders (sickle-cell disorders and thalassemias) were originally characteristic of the tropics and subtropics but are now common worldwide due to migration. [2-5] Many of these abnormal variants are of little clinical significance due to the heterozygous state, but when in the homozygous state or combined with other variants, they may give rise to severe disease. As of today, ~1600 Hb variants have been documented, and the majority do not show any clinical manifestation or are asymptomatic. The WHO reported that 7% of the world's population is carriers of hemoglobin (Hb) disorders.[6]

Thalassemia is classified based on its hemoglobin parts, usually either "alpha" or "beta", or based on its severity, which is called trait, carrier, intermedia, or major thalassemia.[7] Haemoglobin comprises four globin chains: fetal haemoglobin (HbF) has two α and two gamma chains (α2γ2), and adult haemoglobin (Hb A) has two α and two ß chains (α2ß2). Genes in the α-globin and ß-globin gene clusters (on chromosomes 16 and 11) control globin-chain production. Due to spontaneous mutation, haemoglobin gene variants are present at low prevalence (carriers 1-1.5/1000) in all sizeable populations. [3,8] They fall into two broad groups- structural variants that change the amino acid sequence and produce an unusual haemoglobin,[9] and thalassemias that lower or abolish production of globin chains.[10]

Carriers of haemoglobinopathies are often asymptomatic or exhibit only mild anaemia, leading to unawareness of their carrier status. However, when both parents are carriers, there is a 25% chance per pregnancy that the child will inherit a severe form of the disease, such as thalassemia major, necessitating regular blood transfusions and iron chelation therapy. There is also a 50% chance the child will be an asymptomatic carrier and a 25% chance of being unaffected. Identifying carriers is crucial for implementing preventive strategies and providing genetic counselling, particularly in the context of family planning.

Beta thalassemia minor has increased HbA2 (4-8%) level with normal to low elevation of HbF. Beta thalassemia major shows markedly elevated HbF (30-95%) with normal to mildly elevated HbA2 level. Delta beta thalassemia and hereditary persistence of fetal Hb (HPFH) are clinically characterized by increased HbF even in adulthood. Hb-Lepore arises due to the crossover of the delta and beta globin genes. Hb D heterozygotes are clinically asymptomatic, and homozygotes have mild symptoms, i.e., mild hemolytic anaemia, mild to moderate splenomegaly.

Hb E trait is diagnosed by the presence of a high HbA2 (E+A2), approximately 30%. Homozygous HbE patients have approximately 90% HbE+A2 with minor elevation of HbF. HbE+A2 levels of 40-60% with marked elevation of HbF are seen in HbE-ß-thalassemias. [11]

Most cases of Hb J are clinically silent and mostly discovered incidentally during routine investigations or in conjunction with other hemoglobinopathies such as thalassemia [12] and sickle cell anemia [13] while investigating this disease. Hb J presents as an elevated P3 peak on high-performance liquid chromatography (HPLC), while thalassemia is detected by the presence of eluted proteins at the retention time between 0 and 1 minutes.[14] P3 peak up to 6% is considered normal, values 6%-12% indicate a suboptimal specimen, and values greater than 15% indicate Hb J. [15]

The term "sickle-cell disease" includes all manifestations of abnormal HbS levels (proportion of HbS>50%). These include homozygous sickle-cell disease (HbSS) and a range of mixed heterozygous hemoglobinopathies (HbS/ß-thalassemia, HbSC disease, and other combinations).[16]

The clinically most frequently occurring Hb variant is HbS, which causes SCD in homozygosity and in combination with a whole range of other common Hb variants, such as HbC, HbD, HbE, HbO-Arab,etc, and in combination with ß-thalassemia. A variety of less common Hb variants in both the α- and β-globin genes may result in a wide spectrum of clinical conditions, such as erythrocytosis, polycythemia, and hemolytic anaemia. [17]

The percentage of the P3 peak on HPLC increases when blood samples are stored for prolonged periods. This peak represents degraded hemoglobin and typically elutes within the retention time window of 1.50 to 1.90 minutes. Several hemoglobin variants, including Hb J-Meerut, Camden, Austin, Fukuyama, and N-Baltimore, may also elute in this P3 region, potentially leading to diagnostic confusion if the sample is not fresh. [18–21]

Ideally, the P3 fraction should be below 10% for accurate reporting of glycated hemoglobin (HbA1c). Higher values may result from post-translational modifications of adult hemoglobin, which occur as hemoglobin degrades over time. This degradation process is accelerated in stored or older blood samples and is further influenced by elevated glucose levels within red blood cells, which promote glycation and subsequent hemoglobin modification.[22]

## Methodology

**Study design:** This was a retrospective cross-sectional study.

**Study setting:** The study was conducted at the Ma Sarada Charitable Dispensary & Pathology Centre, A unit of Ramakrishna Math, Baghbazar, West Bengal.

**Study procedures:** High-performance liquid chromatography (HPLC) analysis done by: Bio-Rad D10 and Tosho G8 machines.

**Study period:** January 2022 to December 2024

**Source of data:** All the patients who voluntarily came to the camps organized by Ma Sarada Charitable Dispensary & Pathology Centre for HPLC test.

**Sample size:** 5000 cases

**Inclusion criteria:** All the HPLC reports with attached history of the patients and complete blood count reports.

**Exclusion criteria:** Patients who have a history of blood transfusion.

## Results

A total of 5,000 individuals were screened for haemoglobinopathies using high-performance liquid chromatography (HPLC). The overall prevalence of haemoglobinopathies was 11.2%, while 89.6% of individuals had normal HPLC patterns (Table 1). Beta-thalassemia trait was the most frequently detected abnormality, accounting for 318 cases (6.36%), followed by haemoglobin E trait in 167 cases (3.34%). Other haemoglobinopathies included HbE disease (0.06%), HbE-thalassemia (0.1%), HbS trait (0.26%), HbS disease (0.06%), and hereditary persistence of fetal haemoglobin (HPFH) trait (0.22%).

**Table 1. Prevalence of different haemoglobinopathies in West Bengal.**

| Diagnosis | No. of patients | % of the patients (n=5000) |
|---|---|---|
| Beta thalassemia carrier | **318** | **6.36** |
| Hb E carrier | 167 | 3.34 |
| Hb E disease | 3 | 0.06 |
| Hb E beta thalassemia | 5 | 0.1 |
| Hb S carrier | 13 | 0.26 |
| Hb S disease | 3 | 0.06 |
| HPHF trait | 11 | 0.22 |
| Normal | 4480 | 89.6 |
| Total | 5000 | 100 |

## District-wise Distribution

The geographic distribution of haemoglobinopathies is detailed in Table 2. The highest number of beta-thalassemia carriers was observed in North 24 Parganas (7.11%), followed by Bankura (6.42%) and South 24 Parganas (7.06%). HbE carriers were predominantly reported in South 24 Parganas (5.59%) and West Midnapore (3.49%). Other abnormalities, including HbS and HPFH, were also mainly reported in North 24 Parganas.

**Table 2- Distribution of the study population in the districts of West Bengal**

| District (Total no. of Participants: n) | Normal Interpretation | | Beta thalassemia carrier | | Hb E carrier | | Other abnormalities | |
|---|---|---|---|---|---|---|---|---|
| | Total | % | Total | % | Total | % | Total | % |
| **North 24 Parganas (n=1646)** | 1454 | 88.34 | 117 | 7.11 | 57 | 3.46 | 18 | 1.09 |
| **Bankura (n=1465)** | 1323 | 90.31 | 94 | 6.42 | 45 | 3.07 | 3 | 0.20 |
| **West Midnapore (n=802)** | 726 | 90.52 | 42 | 5.24 | 28 | 3.49 | 6 | 0.75 |
| **Purulia (n= 495)** | 455 | 91.92 | 27 | 5.45 | 13 | 2.63 | 0 | 0 |
| **South 24 Parganas (n= 340)** | 290 | 85.29 | 24 | 7.06 | 19 | 5.59 | 7 | 2.06 |
| **Hooghly (n=166)** | 156 | 93.98 | 8 | 4.81 | 1 | 0.60 | 1 | 0.60 |
| **Howrah (n=86)** | 76 | 88.37 | 6 | 6.98 | 4 | 4.65 | 0 | 0 |

## Mentzer Index Distribution

The Mentzer index, used to differentiate iron deficiency anemia from beta-thalassemia trait, was calculated for all cases. As shown in Table 3, most individuals with haemoglobinopathies had a Mentzer index below 13, including those with beta-thalassemia trait (mean: 12.8), HbE trait (12.1), and HbS trait (11.6). In contrast, individuals with normal haemoglobin profiles had a significantly higher average Mentzer index of 21.48.

**Table 3- Distribution of Mentzer's Index in different groups of patients and normal subjects**

| Cases | Average Mentzer's Index (MCV in fl/ RBC count $X10^6/mm^3$) |
|---|---|
| Beta thalassemia carrier (n=318) | 12.8 |
| Hb E carrier(n=167) | 12.1 |
| Hb E disease(n=3) | 12.7 |
| Hb E beta thalassemia(n=5) | 12.4 |
| Hb S carrier(n=13) | 11.6 |
| Hb S disease (n=3) | 11.9 |
| HPFH trait (n=11) | 12.0 |
| Normal (n=4480) | 21.48 |

## Hematocrit and RDW Variation

Table 4 summarizes haematocrit and red cell distribution width (RDW) values. In beta-thalassemia carriers, 49.37% had above-average haematocrit, and 57.55% had elevated RDW. Similar trends were observed in HbE and HbS trait carriers, with most cases showing RDW values above average, reflecting anisopoikilocytosis and red cell size variability typical of these disorders.

**Table 4- Distribution of haematocrit and RDW in different groups of patients and normal subjects**

| Cases | Haematocrit(%) value | | | | RDW (%) value | | | |
|---|---|---|---|---|---|---|---|---|
| | Below average | | Above average | | Below average | | Above average | |
| | No. of patients | % of patients | No. of patients | % of patients | No. of patients | % of patients | No. of patient | %of patients |
| Beta thalassemia carrier (n=318) | 161 | 50.63 | 157 | 49.37 | 135 | 42.45 | 183 | 57.55 |
| Hb E carrier(n=167) | 83 | 49.7 | 84 | 50.3 | 61 | 36.53 | 106 | 63.47 |
| Hb E disease(n=3) | 1 | 33.33 | 2 | 66.67 | 1 | 33.33 | 2 | 66.67 |
| Hb E beta thalassemia(n=5) | 2 | 40 | 3 | 60 | 3 | 60 | 2 | 40 |
| Hb S carrier(n=13) | 6 | 46.15 | 7 | 53.85 | 4 | 30.77 | 9 | 69.23 |
| Hb S disease (n=3) | 2 | 66.67 | 1 | 33.33 | 1 | 33.33 | 2 | 66.67 |
| HPFH trait (n=11) | 6 | 54.55 | 5 | 45.45 | 3 | 27.27 | 8 | 72.73 |
| Normal (n=4480) | 2252 | 50.27 | 2228 | 49.73 | 2983 | 66.58 | 1497 | 33.42 |

## Gender Distribution

Out of 2,129 male patients, 240 (11.27%) had haemoglobinopathies, while 280 of the 2,871 female patients (9.75%) were affected (Table 5). This slight male predominance may be due to higher participation in screening camps or sociocultural factors influencing healthcare access.

**Table 5- Sex distribution of study population**

| Diagnosis | Male | | Female | |
|---|---|---|---|---|
| | Total no. of patients | % of patients (n=2129) | Total no. of patients | % of patients (n=2871) |
| **Normal** | 1889 | 88.73 | 2591 | 90.25 |
| **Haemoglobinopathy** | **240** | **11.27** | 280 | 9.75 |

## Discussion

This retrospective cross-sectional study assessed the prevalence and distribution of haemoglobinopathies in a population of 5,000 individuals screened using high-performance liquid chromatography (HPLC) across selected districts in West Bengal, India. The findings revealed that 11.2% of individuals were carriers or affected by a haemoglobin disorder. The most frequent abnormality detected was beta-thalassemia trait (6.36%), followed by haemoglobin E trait (3.34%), with smaller numbers of compound heterozygous conditions such as HbE-β-thalassemia and HbS disease.

The predominance of beta-thalassemia trait observed in this study is consistent with the known genetic burden of this condition in eastern India. Similar prevalence rates have been reported in earlier regional studies [23,25]. The concentration of HbE carriers in districts such as South 24 Parganas and West Midnapore reflects the ethnic and geographic clustering reported in the literature [23,25].

Although less common, haemoglobin S was identified in both heterozygous and homozygous forms, confirming its presence in certain tribal and non-tribal populations within the region [24]. Rare entities such as hereditary persistence of fetal haemoglobin (HPFH) were detected in 0.22% of cases, and three diagnostically challenging cases required correlation with family history and repeat testing, a reminder of the limitations of relying solely on chromatographic methods [15,21].

A slight male predominance was observed in the detection of haemoglobinopathies (11.27% in males vs. 9.75% in females). This may reflect a higher rate of male participation in the screening camps or social influences on healthcare access rather than biological factors [23].

The beta-thalassemia carrier rate found in this study closely aligns with findings from Mondal and Mondal [23], who reported a 4.6% prevalence using HPLC. Singh et al. [24] reported a higher prevalence of beta-thalassemia trait (15.75%) in Western India, suggesting regional variability influenced by ethnic distribution, consanguinity, and awareness levels.

The use of the Mentzer index in differentiating iron deficiency anemia from beta-thalassemia trait was found to be helpful, as most individuals suspected of having thalassemia had a Mentzer index <13, which supports earlier findings [26,27]. This simple, cost-effective parameter remains useful, especially when integrated with HPLC and complete blood count data.

### Implications of study findings

These findings underscore the importance of population-based screening to detect asymptomatic carriers of haemoglobinopathies. Early identification is crucial for appropriate genetic counselling, partner screening, and prevention of homozygous or compound heterozygous offspring. Despite requiring trained personnel, HPLC remains a reliable, reproducible, and rapid tool for detecting a wide array of haemoglobin variants [15,28,29].

However, interpretation of HPLC results can be influenced by pre-analytical factors, particularly sample quality and the presence of nutritional deficiencies, which may alter HbA2 levels and confound diagnosis. Iron deficiency anemia, in particular, may mask elevated HbA2, leading to underdiagnosis of beta-thalassemia trait [20,30].

### Study Limitations

As a retrospective analysis, this study relies on existing records, which may be incomplete or lack clinical correlation. Furthermore, molecular confirmation was not available in cases with ambiguous or rare chromatographic patterns, limiting diagnostic accuracy. The use of camp-based samples may also introduce selection bias, as individuals attending such events may not be representative of the broader population.

### Future Directions

To enhance diagnostic precision and coverage, future studies should include Cohort studies and prospective screening, particularly in antenatal and school settings, and incorporate molecular techniques for definitive diagnosis. Larger-scale community screening programs, especially in high-prevalence districts, would allow for better estimation of disease burden. Expanding the geographic and demographic scope would ensure representation of remote communities that may have different prevalence patterns or barriers to healthcare access.

Assessing the effectiveness of existing screening, awareness, and counseling programs to identify gaps and improve public health interventions is necessary.
Additionally, cost-effectiveness studies comparing HPLC with other modalities such as capillary electrophoresis could guide policy in resource-constrained settings.

## Conclusion

This study highlights a significant burden of haemoglobinopathies, particularly beta-thalassemia trait and haemoglobin E trait, in the population screened. High-performance liquid chromatography (HPLC) proved to be an effective and practical tool for detecting haemoglobin variants. Early identification of asymptomatic carriers through targeted population screening can aid in timely counselling and prevention of severe haemoglobin disorders in future generations. Integration of HPLC into routine screening programs, especially in high-prevalence regions, is recommended.

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