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Original Research Article

Prevalence and Gender Distribution of β-Thalassemia (Trait and Major) Among Patients with Microcytic Hypochromic Anemia: A Cross-Sectional Study from Western India

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Abstract:

Background: β-Thalassemia is one of the most common inherited hemoglobin disorders worldwide and represents a significant public health challenge in India. Because β-thalassemia trait frequently presents with microcytic hypochromic anemia, it is often misdiagnosed as iron deficiency anemia, leading to inappropriate treatment and missed opportunities for genetic counselling. High-Performance Liquid Chromatography (HPLC) has become an established diagnostic tool for the accurate identification of β-thalassemia and other hemoglobin variants. Regional data on the prevalence of β-thalassemia among patients with microcytic hypochromic anemia remain limited in Western India.

Objective: To determine the prevalence and gender distribution of β-thalassemia (trait and major) among patients presenting with microcytic hypochromic anemia and to evaluate the diagnostic utility of HPLC in a tertiary care teaching hospital.

Materials and Methods: A hospital-based cross-sectional study was conducted in the Department of Pathology, Rajasthan University of Health Sciences College of Medical Sciences, Jaipur, from August 2024 to February 2025. One hundred and fifty patients with microcytic hypochromic anemia (MCV <80 fL and MCH <27 pg) were included. Complete blood counts were performed using an automated hematology analyzer, followed by HPLC using the Bio-Rad D-10 system. Statistical analysis was carried out using IBM SPSS version 22. Chi-square test was used to determine associations between categorical variables, and $p<0.05$ was considered statistically significant.

Results: Among the 150 patients studied, β-thalassemia was diagnosed in 75 (50.0%) patients, comprising 63 (42.0%) cases of β-thalassemia trait and 12 (8.0%) cases of β-thalassemia major. Normal HPLC findings were observed in 50 (33.3%) patients, while 25 (16.7%) patients had other hemoglobin variants. Female patients constituted 64.0% of β-thalassemia cases compared with 36.0% males (female-to-male ratio 1.8:1), demonstrating a statistically significant association ($p<0.05$). The highest prevalence was observed among children younger than ten years and adults aged 21–30 years.

Conclusion: β-Thalassemia is highly prevalent among patients presenting with microcytic hypochromic anemia in Western India. HPLC serves as an accurate and reliable diagnostic modality for early identification of β-thalassemia, facilitating timely genetic counselling, antenatal screening, and appropriate patient management. Routine HPLC evaluation should be considered in patients with persistent microcytic hypochromic anemia, particularly in high-risk populations.

Keywords: β-Thalassemia trait, β-Thalassemia major, Microcytic hypochromic anemia, High-performance liquid chromatography, HPLC, Carrier screening.

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Introduction

β-Thalassemia is one of the most common inherited autosomal recessive disorders worldwide, resulting from reduced or absent synthesis of the β-globin chain of hemoglobin. The imbalance between α- and β-globin chain production leads to ineffective erythropoiesis, chronic hemolysis, and varying

degrees of anemia. Clinically, β-thalassemia ranges from the asymptomatic carrier state (β-thalassemia trait) to severe transfusion-dependent β-thalassemia major. Owing to its lifelong clinical implications, requirement for regular blood transfusions in severe cases, and associated healthcare costs, β-thalassemia continues to be a significant public health problem, particularly in low- and middle-income countries.

According to the World Health Organization (WHO), approximately 5% of the global population carries genes responsible for hemoglobin disorders, with nearly 300,000 children born annually with clinically significant hemoglobinopathies. India bears a substantial share of this burden because of its large population, ethnic diversity, and high carrier frequency. The prevalence of β-thalassemia trait in the Indian population is estimated to be 3–4%, although it may exceed 10% in certain communities. States such as Rajasthan, Gujarat, Punjab, and several tribal regions have reported relatively higher carrier frequencies, emphasizing the need for region-specific epidemiological studies and effective screening programmes.

Microcytic hypochromic anemia is one of the most frequently encountered hematological abnormalities in clinical practice. Although iron deficiency anemia remains its leading cause, β-thalassemia trait is an important differential diagnosis, particularly in areas with a high prevalence of hemoglobinopathies. Both conditions present with low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), making differentiation based solely on routine hematological parameters challenging. Consequently, many patients with β-thalassemia trait are mistakenly treated with iron supplements, delaying accurate diagnosis and missing opportunities for carrier detection and genetic counselling.

Accurate diagnosis of β-thalassemia is therefore essential for appropriate patient management and prevention of severe disease in future generations. Laboratory investigations such as complete blood count, peripheral blood smear examination, hemoglobin electrophoresis, capillary electrophoresis, High-Performance Liquid Chromatography (HPLC), and molecular genetic testing are available for diagnosis. Although molecular analysis remains the definitive diagnostic method, its high cost and limited availability restrict its routine use in many healthcare settings. HPLC has emerged as a rapid, automated, sensitive, and reproducible technique for the identification and quantification of HbA_2, HbF, and other hemoglobin fractions. It has become the preferred screening tool for β-thalassemia because of its accuracy, short turnaround time, and ability to detect multiple hemoglobin variants in a single analysis.

Early identification of β-thalassemia carriers has important clinical and public health implications. Carrier detection facilitates genetic counselling, premarital screening, antenatal screening, and prenatal diagnosis, thereby reducing the incidence of transfusion-dependent β-thalassemia major. In addition, early diagnosis prevents unnecessary iron therapy and enables appropriate counselling of affected individuals and their families. Several national and international guidelines recommend targeted screening of high-risk populations as an effective strategy for controlling the burden of β-thalassemia.

Despite the increasing availability of HPLC in tertiary care centres, data regarding the prevalence and demographic distribution of β-thalassemia among patients with microcytic hypochromic anemia remain limited in Western India. Understanding the regional prevalence and gender distribution of β-thalassemia is essential for planning screening programmes, improving diagnostic strategies, and guiding preventive healthcare policies.

Therefore, the present study was undertaken to determine the prevalence and gender distribution of β-thalassemia (trait and major) among patients presenting with microcytic hypochromic anemia at a tertiary care teaching hospital in Western India. The study also aimed to evaluate the utility of High-Performance Liquid Chromatography as a reliable diagnostic tool for the early detection of β-thalassemia.

Materials and Methods

Study Design and Setting: This hospital-based cross-sectional observational study was conducted in the Department of Pathology, Rajasthan University of Health Sciences College of Medical Sciences (RUHS-CMS), Jaipur, Rajasthan, India, over a six-month period from August 2024 to February 2025. The study was designed to determine the prevalence and gender distribution of β-thalassemia among patients presenting with microcytic hypochromic anemia using High-Performance Liquid Chromatography (HPLC).

Ethical Approval: The study protocol was approved by the Institutional Ethics Committee of Rajasthan University of Health Sciences College of Medical Sciences (Approval No. RUHS-CMS/Ethics Comm./2024/338). Written informed consent was obtained from all adult participants and from parents or legal guardians of pediatric patients before enrolment.

Study Population: A total of 150 consecutive patients with microcytic hypochromic anemia who

were clinically suspected of having hemoglobin disorders were enrolled.

Inclusion Criteria

- Patients of any age and either sex.
- Microcytic hypochromic anemia (MCV <80 fL and MCH <27 pg).
- Patients with clinical suspicion or family history of β-thalassemia.
- Children and parents of known β-thalassemia patients undergoing family screening.

Exclusion Criteria

- Blood transfusion within the previous three months.
- Patients unwilling to provide informed consent.
- Inadequate or hemolyzed blood samples.

Laboratory Evaluation: Approximately 5 mL of venous blood was collected in EDTA vacutainers under aseptic precautions.

Complete blood count was performed using the Sysmex XT-4000i automated hematology analyzer (Sysmex Corporation, Kobe, Japan). Parameters recorded included hemoglobin concentration, red blood cell count, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).

Hemoglobin fraction analysis was subsequently performed using the Bio-Rad D-10 High-Performance Liquid Chromatography (HPLC) System based on cation-exchange chromatography. Daily internal quality control procedures were carried out according to the manufacturer's recommendations. HbA, HbA_2, HbF and abnormal hemoglobin fractions were quantified using standard retention times.

β-thalassemia trait was diagnosed by elevated HbA_2 (≥3.9%) in association with microcytic hypochromic red cell indices, whereas β-thalassemia major was diagnosed by markedly elevated HbF with absent or markedly reduced HbA.

Statistical Analysis: Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 22. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were expressed as frequencies and percentages. Associations between categorical variables were assessed using the Chi-square test. A p-value <0.05 was considered statistically significant.

Results

A total of 150 patients with microcytic hypochromic anemia were enrolled in the study. The age of the participants ranged from 2 months to 64 years, with a mean age of 26.4 ± 15.8 years. Females comprised 82 (54.7%) of the study population, while 68 (45.3%) were males.

Prevalence of β-Thalassemia: Among the 150 patients evaluated by HPLC, 75 (50.0%) were diagnosed with β-thalassemia. Of these, 63 (42.0%) had β-thalassemia trait and 12 (8.0%) had β-thalassemia major. Normal HPLC findings were observed in 50 (33.3%) patients, whereas 25 (16.7%) patients showed other hemoglobin variants.

Table 1: HPLC Findings Among Patients with Microcytic Hypochromic Anemia

Diagnosis	Number	Percentage (%)
β-Thalassemia Trait	63	42.0
β-Thalassemia Major	12	8.0
Other Hemoglobin Variants	25	16.7
Normal HPLC Pattern	50	33.3
Total	**150**	**100**

Gender Distribution of β-Thalassemia: Among the 75 patients diagnosed with β-thalassemia, 48 (64.0%) were females and 27 (36.0%) were males, resulting in a female-to-male ratio of 1.78:1.

Of the 63 patients with β-thalassemia trait, 40 (63.5%) were females and 23 (36.5%) were males. Among the 12 patients with β-thalassemia major, 8 (66.7%) were females and 4 (33.3%) were males.

Table 2: Gender Distribution of β-Thalassemia

Diagnosis	Female n (%)	Male n (%)	Total
β-Thalassemia Trait	40 (63.5)	23 (36.5)	63
β-Thalassemia Major	8 (66.7)	4 (33.3)	12
Total β-Thalassemia	**48 (64.0)**	**27 (36.0)**	**75**

A higher proportion of females was observed among patients diagnosed with β-thalassemia. This difference was statistically significant ($\chi^2 = 4.82$; $p = 0.028$).

Age Distribution: The highest prevalence of β-thalassemia was observed in children younger than 10 years (30.7%), followed by young adults aged 21–30 years (22.7%). The association between age

group and β-thalassemia was statistically significant ($\chi^2 = 28.43$; $p = 0.005$).

Table 3: Age-wise Distribution of β-Thalassemia

Age Group (Years)	Number	Percentage (%)
<10	23	30.7
11–20	15	20.0
21–30	17	22.7
31–40	10	13.3
>40	10	13.3
Total	**75**	**100**

Key Findings: Half of the patients presenting with microcytic hypochromic anemia were diagnosed with β-thalassemia. β-Thalassemia trait was the predominant subtype, accounting for more than four-fifths of β-thalassemia cases. Females constituted nearly two-thirds of the β-thalassemia group, and the highest prevalence was observed among children younger than 10 years and young adults aged 21–30 years.

Discussion

β-Thalassemia is one of the most common inherited hemoglobin disorders worldwide and remains a significant public health challenge in India. Early diagnosis is essential for appropriate clinical management, genetic counselling, and prevention of transfusion-dependent β-thalassemia major. The present hospital-based cross-sectional study evaluated the prevalence and gender distribution of β-thalassemia among patients with microcytic hypochromic anemia using High-Performance Liquid Chromatography (HPLC).

In the present study, 50% of patients with microcytic hypochromic anemia were diagnosed with β-thalassemia, of whom 42% had β-thalassemia trait and 8% had β-thalassemia major. These findings indicate that β-thalassemia is an important cause of persistent microcytic hypochromic anemia in our study population. Although iron deficiency anemia remains the commonest cause of microcytosis, β-thalassemia should always be considered, particularly in regions with a high carrier frequency. Reliance solely on red cell indices may lead to delayed diagnosis and inappropriate iron therapy.

The prevalence observed in this study is consistent with previous Indian reports highlighting the substantial burden of β-thalassemia in the country. Colah et al. reported an overall carrier frequency of 3–4% in the Indian population, with much higher prevalence in selected communities. Similar hospital-based studies have demonstrated a high frequency of β-thalassemia trait among patients investigated for unexplained microcytic hypochromic anemia, emphasizing the importance of targeted screening in high-risk populations.

Among the diagnosed cases, β-thalassemia trait constituted 84% (63/75), whereas β-thalassemia major accounted for 16% (12/75). This distribution is expected because carriers are considerably more common than individuals with transfusion-dependent disease. Identification of carriers has major preventive significance, as appropriate counselling and prenatal screening can reduce the incidence of affected births.

A notable finding of the present study was the female predominance, with females accounting for 64% of β-thalassemia cases. Since β-thalassemia is inherited as an autosomal recessive disorder, no biological sex predilection is expected. The observed predominance is therefore more likely attributable to the hospital-based study design, increased evaluation of anemia in women, and routine antenatal screening rather than a true gender difference in disease prevalence. Similar observations have been reported in hospital-based studies, whereas community-based surveys generally demonstrate comparable carrier frequencies among males and females.

The highest prevalence of β-thalassemia was observed among children younger than 10 years, followed by young adults aged 21–30 years. Early diagnosis in children facilitates timely clinical management, family screening, and genetic counselling. Detection of carriers in young adults is equally important because it supports premarital and preconception counselling, thereby reducing the likelihood of births affected by β-thalassemia major.

HPLC proved to be a reliable and efficient diagnostic technique in the present study. Compared with conventional hemoglobin electrophoresis, HPLC offers rapid automated analysis, excellent reproducibility, and accurate quantification of HbA_2 and HbF, making it particularly useful for diagnosing β-thalassemia trait. Its ability to identify multiple hemoglobin fractions in a single run makes it an ideal screening tool in routine clinical practice and large-scale carrier detection programmes.

Approximately one-third of patients had normal HPLC findings despite presenting with microcytic hypochromic anemia. These patients most likely

represented iron deficiency anemia, anemia of chronic disease, or α-thalassemia trait, highlighting the need to interpret HPLC findings in conjunction with clinical history, complete blood count, peripheral smear, and iron studies. Where indicated, molecular testing may further improve diagnostic accuracy.

The findings of this study reinforce the need for routine HPLC screening in patients with persistent microcytic hypochromic anemia, particularly in regions with a high prevalence of β-thalassemia. Early identification of carriers enables timely genetic counselling and implementation of preventive strategies, ultimately reducing the burden of transfusion-dependent β-thalassemia.

Strengths and Limitations: A major strength of this study is the use of standardized HPLC for diagnosing β-thalassemia in a tertiary care setting, providing contemporary regional data from Western India. However, the study was limited by its single-centre, hospital-based design, which may not represent the true community prevalence. Molecular confirmation of β-globin mutations was not performed, and serum ferritin and iron studies were unavailable for all patients, limiting differentiation between iron deficiency anemia and coexisting β-thalassemia trait.

Conclusion

β-Thalassemia is a common inherited cause of microcytic hypochromic anemia in Western India, with β-thalassemia trait accounting for the majority of cases. HPLC is a rapid, reliable, and effective diagnostic tool for identifying β-thalassemia and should be incorporated into the routine evaluation of patients with persistent microcytic hypochromic anemia. Early diagnosis, carrier screening, and genetic counselling, particularly among adolescents, premarital couples, antenatal women, and first-degree relatives of affected individuals, are essential strategies for reducing the burden of transfusion-dependent β-thalassemia in India. Future multicentric studies incorporating molecular confirmation are warranted to further strengthen the epidemiological evidence.

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

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