OPEN ACCESS

DOI: 10.59736/IJP.23.03.955

ORIGINAL ARTICLE

Hematologic profiling and spectrum of beta-thalassemia trait in reproductive-age groups

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ABSTRACT

Background: Beta thalassemia trait (BTT) is a common inherited hemoglobinopathy in Pakistan. These individuals remain asymptomatic and can pass this gene to their offspring, leading to an increased burden of disease, especially in a resource-limited setting. Our objective is to determine the frequency of BTT among individuals aged 15–49 years using CBC and HPLC parameters.

Methods: It is a retrospective cross-sectional study conducted in the central lab of Dr. Ruth K. M. Pfau Civil Hospital, Karachi. Patients whose High-Performance Liquid Chromatography (HPLC) and CBC tests were done between June 2022 to June 2024 were included. All patients of the reproductive age group (15-49 according to WHO) are included. Different CBC parameters such as Hb, Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Hct, along with the percentage level of different Hb variants like HbA2, HbF, HbS, HbC, HbD, and HbE.HbA2 were analysed. Statistical Package for Social Sciences version 25 (SPSS) was used for statistical data analysis.

Results: Study analyzed 176 patients, consisting of 101 females (57.4%) and 75 males (42.6%). Among these, 49 patients exhibited abnormal HPLC results while 127 had normal results. The frequency of BTT was higher in females than in males. Among the three age groups, 15-24, 25-34, and 35-49 years, the highest incidence of BTT was seen in the 25-34 age range, where 20 cases were identified. It was also noted that individuals with BTT tend to have higher average hemoglobin and hematocrit levels, along with lower MCV levels compared to normal individuals**.**

Conclusion: Frequency of BTT is high in the reproductive age group. Significant differences are observed in hematological indices between BTT and normal individuals, especially in MCV, MCH, and RBC counts. HbA2 levels >3.5% on HPLC proved to be a reliable marker for diagnosing BTT.

Key Words: Beta Thalassemia, Hemoglobinopathies, Microcytosis, Reproductive Age

This article may be cited as: Abbas H, Ahmed N, Fareed N, Urooj I, Anwer G, Barkah A. Hematologic profiling and spectrum of beta-thalassemia trait in reproductive-age groups. Int J Pathol; 23(3):152-8. https://doi.org/10.59736/IJP.23.03.955

Introduction

Hemoglobinopathies such as thalassemia, sickle cell anemia, and hemoglobin E disease are believed to affect 7% of the world population, with an estimated population of 225 million.(1) Pakistan is home to approximately 5000 children who receive a diagnosis of the Beta Thalassemia

trait (BTT)each year. (1) The frequency of BTT in the country ranges from 5.0-7.0%. Hemoglobinopathies, specifically BTT, lack obvious clinical symptoms and remain undetectable despite characteristic hematological parameters, including hypochromia, Microcytosis, and raised hemoglobin A2 (HbA2) levels.(1)

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Nonetheless, the prevalence of asymptomatic carriers is high and detected incidentally in great part by laboratory screening with Complete Blood Count (CBC) and High-Performance Liquid Chromatography (HPLC).(1) Using automated analyzers and HPLC has improved population screening program accuracy and efficiency. Given the high frequency of consanguineous marriages and low knowledge of carrier status in Pakistan, focused screening of those in the reproductive age range provide a chance for early identification and intervention.(1)Using standard laboratory data, this study estimates the incidence and hematological profile of beta-thalassemia trait among tertiary care hospital patients receiving CBC and HPLC testing. Common hereditary hemoglobinopathy in Pakistan, beta-thalassemia trait (β-TT), has a carrier frequency approximated at 5–7 %.(4) Most people with BTT are asymptomatic and are usually found just accidentally during normal blood tests. Early discovery, however, is absolutely vital in stopping thalassemia major from spreading by appropriate genetic counseling and reproductive planning.(5) Detection of other aberrant hemoglobin patterns also depends critically on laboratory screening instruments like Complete Blood Count (CBC) and High-Performance Liquid Chromatography (HPLC). While HPLC directly quantifies hemoglobin variations, such as HbA2, HbF, HbS, HbE, and HbD, CBC offers indirect information through red cell indices including mean corpuscular volume (MCV) and mean corpuscular hemoglobin(MCH).(7) Despite the high burden of thalassemia in Pakistan, population-level carrier screening remains sporadic, especially among individuals of reproductive age. This underscores the need for targeted carrier detection in this demographic. Understanding the distinguished haematological profile of BTT carriers and discriminating them from normal individuals using economically affordable and accessible investigations like CBC and HPLC can predict early identification and warrant further investigations of family members. These findings support the inclusion of routine premarital or antenatal screening programs for future public health policies aimed at reducing the incidence of thalassemia major. Our objective is to determine the frequency of beta-thalassemia trait and other Hemoglobinopathies among individuals aged 15–49 years using CBC and HPLC parameters, and to compare hematological indices between individuals with normal and abnormal HPLC findings — with a particular focus on those diagnosed with beta-thalassemia trait.

Methods

This retrospective cross-sectional study was based on the available data from the central lab Dr. Ruth K. M. Pfau Civil Hospital Karachi of patients whose High-Performance Liquid Chromatography (HPLC) and CBC tests were done between June 2022 to June

2024. Ethical clearance was obtained from Dow University of Health Sciences Institutional Review Board via letter number IRB-3646/duhs/exemption/2024/274. In this study all patients of the reproductive age group, which according to WHO is defined as ages between 15 to 49 years, are included, and All patients with incomplete data and a recent history of blood transfusions within 3 months were excluded. Complete blood count (CBC) was performed on XN 1000 analyzer® and then HPLC was performed on Array analyzer®. The extracted data from the patient's records comprised different CBC parameters, such as Hb, Mean Cell Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC) and Hct along with the percentage level of different Hb variants like HbA2, HbF, HbS, HbC, HbD, HbE. HbA2 value >3.5% was considered as a cut off point for beta thalassemia trait. Statistical Package for Social Sciences version 25 (SPSS) was used for statistical analysis of data.

Result

This study includes a total of 176 patients with 101 females (57.4%) and 75 males (42.6%). *Table I* shows the Demographic and laboratory characteristic of our study population including the complete blood count (CBC) parameters, including hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Among all patients, 49 individuals exhibited abnormal HPLC results, while 127 had normal results.

Table I: Demographic and laboratory characteristic of study population (n=176)

Parameter	Value
Age Range (years)	15 – 49
Sex Distribution	
Male	101 (57.4%)
Female	75 (42.6%)
Hb (g/dL)	9.29 g/dL
Hct (%)	32.29%
MCV (fL)	76.24 fL
MCH (pg)	22.48 pg
MCHC (g/dL)	28.94 g/dL

Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RBC: Red Blood Cell Count.

The HPLC results were categorised into beta thalassemia trait, Hemoglobin S , Hemoglobin E, and Hemoglobin D. The most common hemoglobinopathy identified was the beta thalassemia trait, diagnosis being based on HbA2 levels of more than 3.5%. This condition was particularly prevalent in the age group of 25 to 34 years, while other hemoglobinopathies, such as HBS, HBD, and HBE, were observed at lower frequencies. Table 2 shows distribution of Hemoglobinopathies by Age Group.

Table 2: Distribution of Hemoglobinopathies by Age Group

Age Group (years)	β-Thalassemia Trait (BTT)	β-Thalassemia Major (BTM)	HbS	HbE	HbD	Normal
15 – 24	17	–	1	1	–	59
25 – 34	20	–	–	–	–	45
35 – 49	9	–	–	–	1	23

Dashes (–) indicate no cases recorded.

A comparison of different CBC parameters between normal individuals and beta thalassemia trait is shown in Table 3.

Table 3: Comparison of Hematological Parameters Between Normal Individuals and β-Thalassemia Trait

Parameter	Normal Individuals (Mean ± SD)	β-Thalassemia Trait (Mean ± SD)	P-value
Hb (g/dL)	9.02 ± 3.20	10.15 ± 2.05	0.007
Hct (%)	31.59 ± 9.63	34.63 ± 7.17	0.026
RBC ($\times 10^6$/µL)	4.03 ± 1.31	5.39 ± 1.42	0.00
MCV (fL)	79.87 ± 14.44	66.65 ± 9.76	0.00
MCH (pg)	23.57 ± 5.76	19.61 ± 3.27	0.00
MCHC (g/dL)	28.83 ± 3.30	29.24 ± 1.55	0.272

Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, RBC: Red Blood Cell Count. P value of <0.05 is considered significant

Discussion

Our study evaluated the demographic and hematological characteristics of 176 individuals aged between 15 and 49 years and aimed to observe the distribution of red cell indices in a relatively young population of reproductive age group. The gender distribution exhibited a slight female predominance, with 57.4% females and 42.6% males, which aligns with several community-based screening programs for hemoglobinopathies where females are slightly overrepresented(4). However, study conducted by shaukat et al reported equal prevelance of trait among male and female.(2) In our study higher prevelance of beta thalassemia trait in female is possibly due to antenatal screening and greater healthcare engagement by women in reproductive age groups.

The assessed hematological parameters showed a microcytic, hypochromic pattern with mean Hb of 9.29 g/dL, which is well below the normal reference range, indicating a significant burden of anemia in Beta thalassemia carriers. Shakoor et al (6) and Rashwan et al (11), also reported high burden of anemia in beta thalassemia trait population.(5-6) Accompanying low hematocrit (32.29%) and reduced MCV (76.24fL) further reinforce the predominance of microcytosis in the study population. The decreased MCH and MCHC confirm that the anemia is not only due to decreased red cell size but also reduced hemoglobin content per cell—an overall hypochromic picture. In our cohort, on high-performance liquid chromatography (HPLC), 49 individuals (27.8%) exhibited abnormal hemoglobin profiles, while the remaining 127 (72.2%) had normal profiles. This is a clinically significant finding and suggests a relatively high prevalence of Hemoglobinopathies or hemoglobin variants in our population. Considering the demographic composition and age range, such a proportion of abnormal HPLC findings raise the possibility of inherited hemoglobin disorders like β-thalassemia trait, α-thalassemia, sickle cell trait, or other structural hemoglobin variants. These results underscore the importance of early screening programs, especially in areas with high consanguinity or in populations with known genetic predispositions.

Beta thalassemia trait found to be the commonest hemoglobinopathy among reproductive age groups, having HbA2 levels exceeding 3.4 percent. Elevated HbA2 levels are a hallmark of this condition, as they result from reduced beta-globin chain production and increased delta-globin. (5) Our data is categorized into three age groups: 15-24, 25-34, and 35-39 years. Among the age groups,

Beta Thalassemia trait was most prevalent in the 25-35 age group with a total count of 20 cases, followed by 17 cases from the age group 15-25, and the least cases of 9 from the age group 35-49. These finding are consistent with previous studies, which have shown that thalassemia traits are often detected in young adults, typically between the ages of 20 and 30 years, due to routine screening or clinical symptoms appearing in this age group (6-7).However, the young age bracket of the cohort suggests that findings here may have significant implications for reproductive counseling and carrier screening strategies. For example, identification of asymptomatic carriers in the 15–24 age group can facilitate genetic counseling before marriage or conception, thereby aiding in the prevention of severe Hemoglobinopathies in offspring. The trend of decreasing normal hemoglobin levels with age, from 59 cases in 15-24, to 23 cases in 35-49 group suggests age-related changes with Hemoglobinopathies, malnutrition or undiagnosed issues. In this study, when CBC parameters of normal individuals compared with individuals with beta thalassemia trait, we found that the mean Hb and hematocrit levels were higher in beta thalassemia trait patients compared to normal individuals, and the mean MCV was low in beta thalassemia patients, whereas HbA2 Level was greatly increased in beta thalassemia individuals. Screening of the population with hematological indices and peripheral smear examination in high-prevalence areas is a cheap and easy way to bring light to the beta-thalassemia trait patients, who can then be referred for HB electrophoresis for definitive diagnosis.

Our results strongly suggest a higher carrier detection rate in the early reproductive ages due to targeted screening during antenatal visits or marriage-related follow-ups, further emphasising the importance of premarital and preconception programs for genetic counselling and prevention of the incidence of severe Thalassemia syndrome. Hb E and HbS were rarely detected, with only one case in the 15-24 and 25-35 age groups, with no such cases reported in older groups, which indicate either a low prevalence of these variants in the specific age group or regional genetic differences. The single case of Hb D in the 35-49 age group suggests that this variant is rare but may increase in the future.

The absence of Beta thalassemia Major BTM cases in the data suggests effective prenatal screening or low prevalence, but further vigilance is required as Pakistan is home to approximately 5000 children who receive a diagnosis each year. (16) Identification of carriers is an essential part of prevention programs. Although a nationwide survey is required to know the real burden of disease, studies conducted on a smaller number of subjects can be useful to estimate the burden of disease, particularly in a country with limitations of resources and a lack of efficient primary health infrastructure.(17) These results have broad public health implications. First, they support the incorporation of red cell indices and HPLC screening in routine health check-ups, especially in pre-marital and antenatal contexts. Second, the findings can inform policymakers to implement or strengthen targeted genetic counseling and education programs in schools and primary care centers.

Furthermore, comparing our findings with published literature will help elucidate the regional and ethnic variability in hemoglobinopathy prevalence and the typical hematologic profile of carriers. For example, studies from India, Pakistan, and parts of Africa have reported similar hematological trends in carrier populations,

while Western cohorts typically report lower rates of Hemoglobinopathies unless enriched for immigrant populations.

Conclusion

The study highlights increased frequency of beta-thalassemia trait (BTT) among individuals of reproductive age. Significant differences are observed in hematological indices between BTT and normal individuals, especially in MCV, MCH, and RBC counts. HbA2 levels >3.5% on HPLC proved to be a reliable marker for diagnosing BTT.

Limitations: This is a Retrospective and single centre study. We excluded patients with incomplete data so there is selection bias. No genetic testing done which can validate the HPL Cresults.

Future Recommendations:

Multicenter and prospective study on the same topic so that results may be generalized. Molecular genetic testing is required for confirmation. Targeted screening programs, especially among individuals of reproductive age.

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HISTORY	
Date received:	29-08-2025
Date sent for review:	05-09-2025
Date received reviewers comments:	16-09-2025
Date received revised manuscript:	19-09-2025
Date accepted:	25-09-2025

CONTRIBUTION OF AUTHORS	
AUTHOR	**CONTRIBUTION**
Conception/Design	HA,NA,NUF
Data acquisition, analysis and interpretation	HA,IU,BA
Manuscript writing and approval	HA,IU,GA

All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.