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ORIGINAL ARTICLE

Use of red cell indices in identifying beta thalassemia trait in undergraduate medical students-a pilot study.

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ABSTRACT… Objective: To determine the prevalence of beta thalassemia trait (BTT) among undergraduate medical students using red cell indices in a pilot study. **Study Design:** Cross-sectional descriptive study. **Setting:** Rashid Latif Khan University (RLKU) Medical College. **Period:** Over one year, February 2024 to February 2025. **Methods:** A total of 100 undergraduate medical students were included. Complete blood count (CBC) was performed for all participants. Anaemia was defined according to World Health Organization criteria (hemoglobin <13 g/dL in males and <12 g/dL in females). Anaemic students were further evaluated using red cell indices (MCV, MCH, RDW, RBC count). Mentzer Index (MCV/RBC) was calculated, and values <13 with elevated RBC count were considered suggestive of BTT. Suspected cases underwent hemoglobin electrophoresis using capillary electrophoresis for confirmation. **Results:** The mean age was 20 ± 2 years, with 40% males and 60% females. Anaemia was present in 23% students. Four students (17.4% of anaemic group) had a Mentzer Index <13 with raised RBC count. Hemoglobin electrophoresis confirmed BTT in 2 cases, giving a prevalence of 2%. The positive predictive value of Mentzer Index was 50%. The estimated prevalence was 2% (95% CI: 0.24%–7.0%). **Conclusion:** In this pilot study, red cell indices appear to be a cost-effective screening tool for identifying suspected BTT cases. Larger studies are required to validate these findings.

Key words: Beta Thalassemia Trait, Mentzer Index, MCV, MCH, RDW, RBC Count.

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INTRODUCTION

Thalassemia is a hemoglobinopathy in which there is reduced or absent synthesis of beta globin chains leading to deficiency of beta globin chain synthesis resulting in anemia.[1] In areas with a high carrier frequency, like Pakistan and various Mediterranean and South Asian nations, where carrier rates can approach 5-7% of the overall population, the Thalassemia trait—which is frequently clinically silent—becomes extremely important.[2] Early and reliable identification of beta thalassemia trait carriers is critical for genetic counselling and national prevention programmes aiming at lowering the prevalence of beta thalassemia major, a severe, transfusion-dependent anemia.[3]

Thalassemia leads to growth retardation, pallor, weakness, jaundice, bulging abdomen with enlarged liver and spleen, black urine, deformed facial bones, and failure to thrive. Extramedullary erythropoiesis combined with compensatory enlargement of the erythroid marrow can cause lengthy bone abnormalities and common craniofacial alterations. These features are mainly seen in thalassemia major, whereas beta thalassemia trait is usually asymptomatic[4] Lab features include microcytic, hypochromic anemia with vast variation in red cell indices.[4]

Beta thalassemia trait and iron deficiency anemia are the two most common causes of microcytic hypochromic anemia in our population. Differentiating between the two is clinically important, as their management differs significantly. Red cell indices have been widely used as an initial screening tool for this purpose.[5] In the thalassemia trait, both the MCV and MCH indices are decreased with increased RBC count and high retic count. RDW may be normal or mildly increased in BTT, unlike iron deficiency anemia where it is significantly raised.[6] There are other causes of Microcytic hypochromic anemia in our population which can confound the presence of thalassemia; the commonest being iron deficiency anemia due to poor dietary intake and

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recurrent parasitic infestations.[7]

Hemoglobin electrophoresis and molecular analysis are the gold standards for identifying hemoglobinopathies, but in healthcare settings with limited resources, these techniques are sometimes expensive, technically complex, and not generally accessible.[8,9] Consequently, inexpensive, and accessible laboratory screening methods hold critical roles in initial case detection.[9] Among these, automated complete blood count (CBC)-derived red cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), red cell distribution width (RDW), and red blood cell count (RBC), are frequently accessible and have been thoroughly investigated for their potential to differentiate BTT from other microcytic anemia causes.[10]

To increase the sensitivity and specificity for BTT screening, several discriminating indices and formulas based on red cell properties have been suggested and verified in various populations. These include the Mentzer index (MCV/RBC), Shine and Lal index, England, and Fraser index, and RDW index.[11] These indicators aid in distinguishing iron deficiency anemia (IDA), in which microcytosis is associated with a low RBC count, from BTT, which is characterized by microcytosis with a relatively conserved or raised RBC count.[12] More recently, red cell index-based machine learning algorithms have shown increased diagnostic accuracy, which further expands the potential of hematological parameters as trustworthy screening tools.[13]

Screening undergraduate medical students represents an important public health initiative given their future roles as healthcare providers and their potential to influence awareness and prevention strategies within communities. Validating the diagnostic performance of red cell indices in such a population helps define cost-effective, scalable screening protocols, minimizing the need for expensive confirmatory testing at the initial stage. Additionally, using red cell indices increases screening acceptability by avoiding invasive methods like venipuncture in populations opposed to blood sample.[14]

Despite the promise of red cell indices, challenges remain related to variability in cutoff values across ethnic groups and coexisting nutritional deficiencies affecting red cell parameters, necessitating locally validated diagnostic criteria. Recent research highlights the importance of integrating laboratory and clinical data with red cell indices for thorough screening methods.[15]

Thalassemia is a genetic disorder which can only be managed with extremely costly healthcare, however if the carrier/trait is detected, the disease can be prevented from passing on through next generations and hence burden on resource-constrained healthcare system can be reduced.

RATIONALE

The present study is aimed at identifying beta thalassemia trait in undergraduate medical students by Red Cell Indices. The results of this study will be utilized in giving recommendations about cost-effective screening tools for initial workup of thalassemia trait and will help in better diagnosis and preventive action plan.

METHODS

This cross-sectional descriptive study was conducted at Rashid Latif Khan University (RLKU) Medical College over a period of one year, after approval of the research synopsis from February 2024 to February 2025. Students enrolled in 1st year and 2nd year MBBS at RLKU Medical College were included in the study.

The sample size was calculated using the formula:

$n = Z^2 \times p \times (1 - p) / d^2$

Assuming a prevalence (p) of 3% based on global carrier frequency of β-thalassemia, a confidence level of 95% (Z = 1.96), and a margin of error (d) of 3%, the calculated sample size was 93. This was rounded up to 100 participants to improve study reliability.

Inclusion Criteria

- MBBS students of 1st and 2nd year
- Students who provided written informed consent

Exclusion Criteria

- Students who did not consent to participate

Data Collection and Laboratory Methods

Venous blood samples were collected from all participants under aseptic conditions in the Pathology Laboratory. Complete blood count (CBC) was performed for all samples.

Students found to be anemic were further evaluated for red cell indices including MCV, MCH, RDW, and RBC count**.** Due to feasibility constraints, further evaluation using red cell indices was limited to students identified as anaemic. This approach limits the study's ability to detect non-anaemic carriers and may underestimate the true prevalence of beta thalassemia trait.

Mentzer's Index was calculated using the formula:

$$\text{Mentzer Index} = \frac{\text{MCV (fL)}}{\text{RBC count (million/µL)}}$$

Students with a Mentzer Index < 13 and raised RBC count were shortlisted for further evaluation. The Mentzer Index was selected for screening due to its simplicity, ease of calculation, and widespread use in differentiating beta thalassemia trait from iron deficiency anemia in resource-limited settings. It requires only two routinely available parameters (MCV and RBC count), making it practical for large-scale screening. Although other indices such as the Shine and Lal index have been reported to have higher diagnostic accuracy in certain studies, the Mentzer Index remains one of the most used and validated screening tools in clinical practice.

Blood samples of shortlisted students were recollected and subjected to hemoglobin electrophoresis to confirm thalassemia trait.

VARIABLES

- Hematological parameters: Hb, MCV, MCH, RDW, RBC count
- Mentzer Index
- Hemoglobin electrophoresis results

Statistical Analysis

Data were entered and analyzed using SPSS version 26.0 (IBM Corp., USA). Results were expressed as frequencies, percentages, and mean values. Graphical representation was done using bar charts to compare hematological parameters between normal students and those suspected of beta-thalassemia trait.

Ethical Considerations

Ethical approval was obtained from the Institutional Review Board / Research Advisory Committee of RLKU Medical and Dental College (Approval No. RLKU.IRB-008/23, dated 30th November 2023). Informed consent was obtained from all participants.

RESULTS

A total of 100 undergraduate medical students were included in the study. The mean age of participants was 20 ± 2 years, with 40 (40%) males and 60 (60%) females.

Out of 100 students, 23 (23%) were found to be anaemic based on World Health Organization (WHO) criteria (hemoglobin <13 g/dL in males and <12 g/dL in females). Among the anaemic students, 9 (39.1%) were males and 14 (60.9%) were females. These students were further evaluated using red cell indices.

Mean values of red cell indices for the normal and suspected beta thalassemia trait groups are presented in Table-I

TABLE-I

Comparison of mean (±SD) hematological parameters between normal and suspected beta thalassemia trait groups

Parameter	Normal Group (n=96)	Suspected BTT (n=4)
MCV (fL)	88.28 ± 7.51	69.75 ± 6.29
MCH (pg)	29.04 ± 2.99	21.75 ± 1.71
RBC ($\times 10^6$/µL)	4.58 ± 0.49	6.04 ± 0.20
RDW (%)	13.90 ± 1.39	16.10 ± 1.56

Normal Group = everyone except the 4 "BTT-suspected" cases

- Suspected BTT (n=4) = the 4 lowest Mentzer index values, where Mentzer index = MCV / RBC (a common screening approach for suspected thalassemia trait)

Among the anaemic group, 4 (17.4%) students demonstrated a Mentzer Index <13 along with an elevated red blood cell (RBC) count, suggestive of possible beta thalassemia trait. These cases represented 4% of the total study population and were shortlisted for confirmatory testing.

Hemoglobin analysis was performed using capillary

electrophoresis (Sebia, Capillary 3 OCTA) that confirmed beta thalassemia trait in 2 out of the 4 suspected cases, with raised HbA2 levels (4.7% and 5.7%). This resulted in an observed prevalence of 2% in the overall study population.

Peripheral blood smear examination of the shortlisted cases showed that the two confirmed beta thalassemia trait cases exhibited microcytic hypochromic red cells with anisocytosis and poikilocytosis, along with the presence of occasional target cells and elliptocytes. These morphological findings were consistent with beta thalassemia trait.

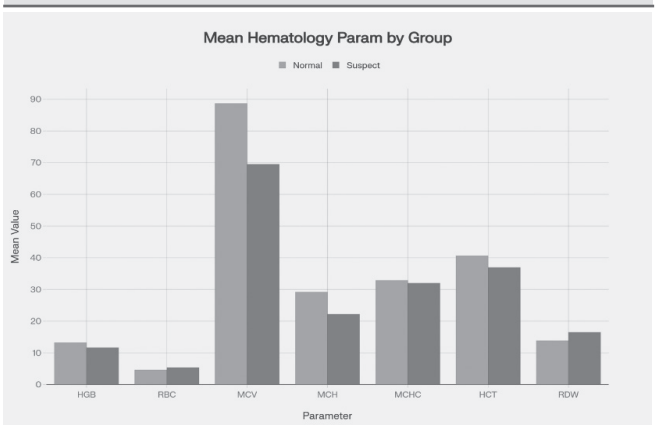
Iron studies were not performed for all participants; however, serum ferritin was assessed in the shortlisted suspected cases. Among them, the two individuals confirmed to have beta thalassemia trait showed normal serum ferritin levels, whereas one of the two cases not confirmed as BTT was found to have iron deficiency.

The positive predictive value (PPV) of the Mentzer Index in this study was 50%. The prevalence of beta thalassemia trait was estimated at 2% (95% confidence interval: 0.24%–7.0%). This wide confidence interval overlaps with the reported national prevalence (5–7%), indicating limited precision due to the relatively small sample size.

P-value calculated using one-sample proportion (binomial) test. A p-value < 0.05 was considered statistically significant.

FIGURE-1

Comparison of mean hematological parameters between normal students and those suspected of beta thalassemia trait.



The suspected group (n=4) showed lower mean MCV and MCH values with relatively higher RBC counts compared to the normal group. Parameters are expressed as mean values: Hb (g/dL), RBC ($\times 10^6$/µL), MCV (fL), MCH (pg), MCHC (g/dL), HCT (%), and RDW (%).

DISCUSSION

Although the prevalence is not very high, but the point of significance is that how a simple test can help screen a population that can lead to a better and easy approach towards diagnosis and preventive strategies.

The screening in this study was done using red cell indices (e.g., MCV, MCH, RBC count, RDW) that helped in detecting beta-thalassemia trait (BTT) among undergraduate medical students. In the present study, only anaemic students were further evaluated using red cell indices. This approach may have led to under-detection of beta thalassemia trait, as carriers—particularly males—can have hemoglobin levels within the normal range. Consequently, individuals with beta thalassemia trait but without anemia may not have been identified, potentially resulting in an underestimation of the true prevalence.

Various studies have been conducted to determine the prevalence of Beta Thalassemia trait in Pakistan. In a study in the twin cities, on a sample size of 60, the prevalence came out to be 2.3% which is close to the results obtained in our study.[16]

A study conducted in a semi-urban population of Karachi reported a substantially higher prevalence of beta thalassemia trait (7.2%) using a similar screening approach based on the Mentzer Index.[17] This is notably higher than the 2% prevalence observed in our study. Several factors may account for this difference. Firstly, the Karachi study included a larger sample size (n=578), resulting in a narrower confidence interval and more precise prevalence estimate. In contrast, the present study was conducted on a smaller cohort of 100 participants, leading to wider confidence intervals and reduced precision.

Secondly, differences in study population may have

contributed to the variation. The Karachi study sampled a semi-urban general population, whereas our study was limited to undergraduate medical students, a relatively homogeneous and potentially lower-risk group with different sociodemographic characteristics.

Thirdly, in our study, only anaemic individuals were further evaluated using red cell indices, which may have led to under-detection of beta thalassemia trait, as carriers can have normal hemoglobin levels. This methodological difference could have contributed to the lower observed prevalence. Additionally, regional genetic variability and differing rates of consanguinity may also influence carrier frequency across populations in Pakistan.

Therefore, the lower prevalence observed in our study should be interpreted with caution, and larger population-based studies are required for accurate estimation.

In Pakistan β-thalassemia trait frequency ranges between 5.0–7.0% according to a review article on Thalassemia in Pakistan.[18]

The prevalence of β-thalassemia trait (BTT) was found to be 0.69% (26/3755) in a study done in in the Adult Population of Al Majma'ah, Saudi Arabia. This is much lower as compared to the results obtained in our research. However, there are many possible reasons for the differences which include genetic variations, environmental factors, and sample size differences.[19]

According to the Global Burden of Disease (GBD) Study 2021, thalassemia remains a significant global health burden, with a substantial number of individuals affected worldwide (20) Our study results match with global prevalence of Thalassemia trait however the results may vary if screening is done in rural areas of Pakistan where cousin marriages are more common.

Early detection of BTT utilizing red cell indices—such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and red blood cell (RBC) count—is critical in screening programs because it allows for the early identification of carriers within the community. This early detection is crucial because it not only promotes better genetic counselling and informed reproductive choices, but it also aids in the implementation of prophylactic interventions to lower the birth prevalence of severe thalassemia syndromes.

Differentiating BTT from iron deficiency anemia (IDA) is critical in clinical practice. Both disorders are frequently associated with microcytic, hypochromic anemia and may appear identical on a basic blood count. However, their management approaches are fundamentally different. If BTT is misinterpreted for IDA, people may receive unneeded iron supplementation, which has no benefit and can lead to iron overload and toxicity, particularly in thalassemia carriers, whose bodies already collect iron. Conversely, failing to diagnose BTT may preclude adequate counselling and family planning.

In this study, RDW was used as an adjunct parameter in differentiating iron deficiency anemia from beta thalassemia trait. IDA typically shows markedly elevated RDW due to anisocytosis, whereas BTT is associated with relatively uniform microcytosis and normal or mildly increased RDW. However, RDW alone is not definitive and should be interpreted in combination with other red cell indices.[10] In resource-limited settings, RDW may serve as a supportive parameter to differentiate IDA from BTT when iron studies are not routinely feasible, although it cannot replace definitive biochemical testing. The absence of iron studies may have contributed to false-positive Mentzer index results.

Thus, careful interpretation of red cell indices not only aids in the early discovery of BTT and the prevention of thalassemia major births but also ensures that iron therapy is reserved for truly iron-deficient patients, minimizing injury, and optimizing resource allocation.

This study has significant strengths, particularly in its use of a young, educated population—undergraduate medical students—who are more likely to provide accurate histories and dependable follow-up. The use of RBC indices such as MCV and MCH, along with the Mentzer Index (MCV/RBC count), is a simple, cost-effective screening

tool for identifying beta thalassemia trait. A Mentzer Index <13 typically suggests beta thalassemia trait, helping distinguish it from iron deficiency anemia.

Kumar et al. reported that the Shine and Lal index may have superior diagnostic performance compared to the Mentzer Index in differentiating beta thalassemia trait from iron deficiency anemia.[21] However, in the present study, the Mentzer Index was preferred due to its simplicity, minimal parameter requirement, and ease of application in routine clinical settings. While the use of a single index may limit diagnostic accuracy, combining multiple indices could improve sensitivity and specificity, which may be explored in future studies. To get a definitive segregation, none of the indexes can be fully trusted. However, Green and King and Mentzer indices can be used for screening the suspected population to identify patients which may need the specialized tests to confirm the diagnosis to bring this population under proper treatment either for IDA or BTT.[22]

Furthermore, the use of statistical analysis improves the study's validity by allowing for objective examination of correlations and prevalence, even with a potentially small sample size.

The limitation of the study is that the sample size is small that limits the external validity of the study. The estimated prevalence may not reflect the true prevalence of Beta thalassemia in general population. Only anemic students were further evaluated using red cell indices, which may have resulted in under-detection of beta thalassemia trait, as carriers can have normal hemoglobin levels. Although iron studies were performed in suspected cases, they were not done for all participants which limits differentiation between iron deficiency anemia and beta thalassemia trait in borderline cases. Moreover, no confirmatory genetic testing was done, and we are unable to rule out any co-existing conditions like iron deficiency anemia, alpha -thalassemia based on red cell indices and electrophoresis.

To get a better picture of the prevalence of Beta Thalassemia trait in our population, the study can be performed on a larger and more diverse population. Red cell indices may be combined with molecular diagnostics for better accuracy. Further research needs to be done to assess the awareness and genetic counseling needs of medical students regarding beta-thalassemia.

CONCLUSION

Red cell indices are a low-cost and practical screening approach for diagnosing beta thalassemia trait (BTT) that uses common blood indicators such as MCV, MCH, and RBC count. They efficiently detect suspected carriers, particularly in resource-limited settings, minimizing the need for costly investigations. However, because red cell indices alone cannot confirm a diagnosis, additional testing with hemoglobin electrophoresis and genetic analysis is required to distinguish BTT from other illnesses such as iron deficiency anemia and avoid incorrect treatment. Raising awareness and combining screening with genetic counselling enable at-risk couples to make educated reproductive decisions, thereby preventing beta-thalassemia major and reducing disease burden.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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2	**Nadia Mahmood:** Proof reading.
3	**Ayesha Jamil:** Manuscript review.
4	**Namra Mehmood:** Data analysis.
5	**Sher Ali:** Data collection.
6	**Uzair Shahid:** Data collection.