

Evaluation of the diagnostic reliability of Mentzer index for Beta thalassemia trait followed by HPLC

Saxena S.¹, Jain R.^{2*}

DOI: <https://doi.org/10.17511/jopm.2020.i02.03>

¹ Shubhi Saxena, Assistant Professor, Department of Pathology, Smt. B K Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed University, Vadodara, Gujarat, India. <https://orcid.org/0000-0002-0616-5741>

^{2*} Richa Jain, Assistant Professor, Department of Pathology, Smt. B K Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed University, Vadodara, Gujarat, India. <https://orcid.org/0000-0001-8141-2602>

Introduction: The individuals having thalassemia trait usually have an asymptomatic course and have mild microcytic hypochromic anemia. Since the other cause of microcytic anemia is iron deficiency, it is important to differentiate it from thalassemia trait. **Objectives:** This study aims at figuring out the diagnostic value and reliability of the Mentzer index in the differentiation of iron deficiency anemia and thalassemia carrier compared with the results of HPLC. **Methods:** This was a hospital-based retrospective observational study done on 1236 patients of all age groups. Mentzer index of all the patients was calculated and the results were analyzed and further compared with HPLC. **Results:** Out of 1236 patients, 741 (59.9%) patients are of iron deficiency anemia and 495 (40.1%) patients are of β thalassemia trait. Mentzer index was found to be more reliable to detect true positive cases for β thalassemia trait with a sensitivity of 89.0%, specificity of 87.9%. **Conclusion:** Iron deficiency anemia and thalassemia have different effects on blood indices. In resource-poor and developing countries like that of India, it can be used as a screening tool. In doubtful cases, the diagnosis can be confirmed by HPLC.

Keywords: Beta Thalassemia trait, Iron deficiency anemia, Mentzer Index

Corresponding Author

Richa Jain, Assistant Professor, Department of Pathology, Smt. B K Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth Deemed University, Vadodara, Gujarat, India.
Email: dr.richa.rj@gmail.com

How to Cite this Article

Saxena S, Jain R. Evaluation of the diagnostic reliability of Mentzer index for Beta thalassemia trait followed by HPLC. Trop J Pathol Microbiol. 2020;6(2):124-129.
Available From
<https://pathology.medresearch.in/index.php/jopm/article/view/397>

To Browse



Introduction

Microcytic hypochromic anemia is characterized by decreased hemoglobin, PCV, MCV, MCH, MCHC and normal to increased RDW. Microcytic anemia in a case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (Hb)

Synthesis, resulting in microcytosis and hypochromia [1]. It is the most common genetic disorder in the world. WHO reported that 5% of the world's population is thalassemia carriers [2]. Due to migration and intermarriage of different ethnic populations, β thalassemia trait is found in people with no obvious ethnic connection to the disorder.

Manuscript Received
10-01-2020

Review Round 1
20-01-2020

Review Round 2
25-01-2020

Review Round 3

Accepted
29-01-2020

Conflict of Interest
No

Funding
Nil

Ethical Approval
Yes

Plagiarism X-checker
15%

Note



© 2020 by Shubhi Saxena, Richa Jain and Published by Siddharth Health Research and Social Welfare Society. This is an Open Access article licensed under a Creative Commons Attribution 4.0 International License <https://creativecommons.org/licenses/by/4.0/> unported [CC BY 4.0].



While the diagnosis of beta-thalassemia major usually becomes obvious within initial years of life because of progressive anemia, it is children with beta-thalassemia trait who pose a diagnostic dilemma [3].

High-Performance Liquid Chromatography (HPLC) technique is not widely available in India. It is necessary to develop a simple examination method that can be performed at the district health centers. One of the examination parameters that can be used for a thalassemia carrier screening test is the Mentzer index. Mentzer index is an MCV/RBC ratio calculation in which patients with a value of <13 is diagnosed as thalassemia carriers while a value of >13 is found in patients with iron deficiency [4,5].

The aim of this study was to find out the diagnostic value of the Mentzer index and to judge its reliability in differentiating between β thalassemia trait and iron deficiency anemia.

Materials and Methods

Type of study and duration: This is a hospital-based observational retrospective study done from December 2018 to December 2019 for a duration of one year.

In this study, 1236 patients of all age groups were selected. The samples were obtained during the course of routine analysis and collected in EDTA anticoagulant tubes. Thalassemia carrier diagnostic criteria were anemia with MCV <80 fl, MCH <27 pg and HbA₂ fraction >3.5% [6,7,8].

Mentzer index was calculated as the MCV/RBC ratio in which patients with a value of <13 is diagnosed as thalassemia carriers while a value of >13 is found in patients with iron deficiency anemia [4,5].

The diagnosis of iron deficiency anemia was done on the basis of blood picture and iron studies carried out on a five-part cell differential counter and the diagnosis of β thalassemia was done on the basis of increased HbA (>3.5%) levels estimated by BIO-RAD D10 Dual Reader which uses HPLC technique. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index were calculated for each measure as follows:

Sensitivity = $[\text{True positive} / (\text{True positive} + \text{False negative})] \times 100$

Specificity = $[\text{True negative} / (\text{True negative} + \text{False positive})] \times 100$

PPV = $[\text{True positive} / (\text{True positive} + \text{False positive})] \times 100$

NPV = $[\text{True negative} / (\text{True negative} + \text{False negative})] \times 100$

Youden's index = $(\text{Sensitivity} + \text{Specificity}) - 100$

Inclusion criteria

01. Patients already diagnosed with iron deficiency anemia or thalassemia trait on the basis of blood picture, iron studies, and HPLC.
02. Patients of all age groups.

Exclusion criteria

- A) Coexistence of other hematological conditions like autoimmune hemolytic anemia, aplastic anemia or lead intoxication.
- B) Coexistence of β thalassemia trait and iron deficiency anemia in the same patient.
- C) History of blood transfusion in near past

Results

The peripheral blood examination of 1236 patients who were included in this study revealed microcytic hypochromic anemia. The diagnosis of microcytic hypochromic anemia was further confirmed by evaluating the blood samples run through a five-part cell counter which revealed decreased Hb, PCV, MCV, MCH, and MCHC. The confirmation of iron deficiency anemia was done by conducting iron studies to rule out other differential causes. The diagnosis of thalassemia trait was done on the basis of increased HbA₂ on HPLC.

A total of 1236 patients were analyzed, 741 (59.9%) patients are of iron deficiency anemia and 495 (40.1%) patients are of β thalassemia trait (Figure 1).

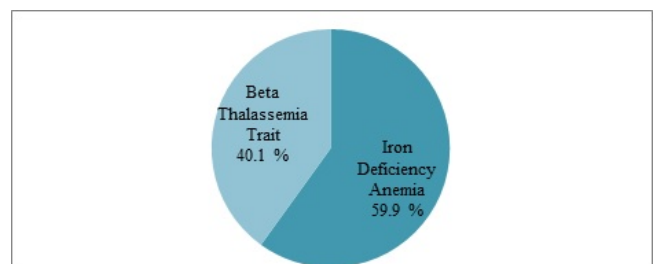


Fig-1: Total number of patients afflicted.

An analysis of the presenting signs and symptoms of patients was also done. The most common clinical feature of patients suffering from iron deficiency

Anemia was found to be fatigue (74.6%), followed by breathlessness (16.1%), irritability (4.4%), anorexia (3.7%) and 0.9% were asymptomatic. It was found that most of the patients suffering from β thalassemia trait were asymptomatic (67.3%). However, some of them presented with fatigue (12.2%), irritability (9.3%), breathlessness (8.7%) and anorexia (2.5%) (Figure 2).

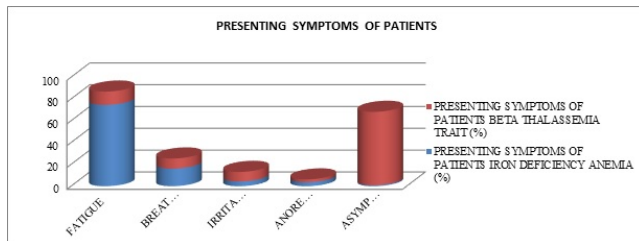


Fig-2: Presenting symptoms of patients

Out of 495 total patients of β thalassemia trait, 441 (89%) patients have Mentzer Index <13 and out of 741 total patients of iron deficiency anemia, 652 (87.9%) patients have Mentzer Index >13 (Table 1).

Table-1: Mentzer Index.

	Mentzer Index >13	Mentzer Index <13
Iron Deficiency Anemia	652	89
Beta Thalassemia trait	54	441

The sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index were calculated for both iron deficiency anemia and β thalassemia trait using the formulae as stated above in the materials and methods. The values have been tabulated below in Table 2:

Table-2: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Youden's Index of iron deficiency anemia and β thalassemia trait.

Parameters for Mentzer Index	Iron Deficiency Anemia	β Thalassemia Trait
Sensitivity (%)	87.9	89.0
Specificity (%)	89.0	87.9
PPV (%)	92.3	83.2
NPV (%)	83.2	92.3

Sensitivity is the probability that a test will indicate 'disease' among those with the disease while specificity is the fraction of those patients without disease who will have a negative test result. Mentzer index was found to be more reliable to detect true positive cases for β thalassemia trait with a sensitivity of 89.0 % whereas it was more specific to pick the true negative cases of iron

Deficiency anemia with a specificity of 89.0 %. The positive predictive values for iron deficiency anemia and β thalassemia trait were 92.3 % and 83.2 % respectively. The negative predictive value for iron deficiency anemia and β thalassemia trait were 83.2 % and 92.3 % respectively. Youden's index was also calculated using the formula: (Sensitivity + Specificity) - 100, which came out to be 76.9.

Discussion

Thalassemia, a disorder caused by the decrease or absence of one or more globin chain synthesis, is the most common genetic disorder in the world. WHO reported that 5% of the world's population is thalassemia carriers [2].

Iron deficiency anemia and β thalassemia trait are among the most common causes of microcytic anemia encountered in India. Distinguishing β thalassemia trait from iron deficiency anemia has important clinical implications because each disease has an entirely different cause, prognosis, and treatment. Children are predisposed to iron deficiency because of dietary insufficiency, growth and helminthic infestations.

On the other hand, β thalassemia trait is usually asymptomatic which is caused by a mutation in one β globin gene. β thalassemia accounts for a major disease load in Southeast Asia, especially in India. In India, the communities which are majorly inflicted are Sindhis, Gujaratis, Punjabis, Prajapatis, Bengalis and Kachchis due to the presence of a higher number of thalassemia trait patients [9].

Misdiagnosis of β thalassemia trait has consequences for potential homozygous offspring leading to β thalassemia disease. The presenting symptoms of patients suffering from iron deficiency anemia and β thalassemia trait are usually similar consisting mostly of fatigue, breathlessness, anorexia, angular stomatitis, and irritability. However, most of the patients of β thalassemia trait remain asymptomatic and present with symptoms in their later age.

The diagnosis of iron deficiency anemia depends upon reduced PCV, MCV, MCH, and MCHC but is confirmed by carrying out iron studies. The classical findings seen in iron deficiency anemia are reduced serum ferritin and serum iron along with increased total iron-binding capacity. The diagnosis of thalassemia is dependent upon the demonstration of increased HbA2 levels in the blood (> 3.5%) on HPLC and mutation analysis [10].

Mentzer WC et al from Turkey assessed 290 children aged 1 - 16 years and used the red blood cell count, RDW and Mentzer index (mean corpuscular volume/red blood cell count ratio) to differentiate β thalassemia trait from iron deficiency anemia. These results indicated that the Mentzer index was the most reliable indicator, with a sensitivity of 98.7% and specificity of 82.3% [11]. In 2009, Ehsani et al showed that the best discrimination index according to Youden's criteria was the Mentzer index (90.1%), followed by the Ehsani et al index (85.5%). In their study, the Mentzer and Ehsani et al were able to correctly diagnose 94.7% and 92.9% of cases, respectively [12]. Similar results (Mentzer index: sensitivity, 90.9%; specificity, 80.3%) were found by Ghafouri et al [13].

Batebi et al [14] reported sensitivity and specificity of the Mentzer index as 86.3% and 85.4% respectively. Some have reported a lower sensitivity of 67% in Mentzer's index while other studies have shown higher sensitivity with this index (82-95%) [15-17].

Recently, there are more than 200 mutations in the β -globin gene that have been found. Differences in gene mutations will cause differences in the severity of clinical signs and symptoms in patients including blood indices, including Hb, RBC, and MCV levels. β^+ mutations in globin chains will cause lower Hb, RBC, and MCV values lower than β^0 mutations [10]. Though the definitive tests for iron deficiency anemia and thalassemia are iron studies and HPLC respectively, it is difficult to perform these tests in all patients having microcytic hypochromic anemia as they are costly. The HbA₂ analysis is however considered to be the gold standard for diagnosing thalassemia. Electronic cell counters have been used to determine red cell indices as the first indicator of TT. The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs. Since 1970, a number of complete blood count indices have been proposed as simple and inexpensive tools to determine whether a blood sample is more suggestive of TT or IDA [12,18,25]. It is for this reason that the Mentzer index as a diagnostic screening tool was studied to differentiate between iron deficiency anemia and thalassemia trait [4]. Mentzer index is an MCV/RBC ratio calculation in which patients with a value of <13 is diagnosed as thalassemia carriers while a value of >13 is found in patients with iron deficiency [4,5]. The diagnosis of

β thalassemia trait involves measuring the HbA₂ concentration of lysed RBCs via HPLC. Several studies have shown that iron deficiency directly affects the rates of HbA₂ synthesis in the bone marrow; therefore, 16-20 weeks of iron therapy should be instituted, after which a repeat serum iron with HPLC is done to confirm improvement in the HbA₂ levels [26].

Mentzer index was found to be more reliable to detect true positive cases for β thalassemia trait with a sensitivity of 89.0 % whereas it was more specific to pick the true negative cases of iron deficiency anemia with a specificity of 89.0 %. The positive predictive values for iron deficiency anemia and β thalassemia trait were 92.3 % and 83.2 % respectively. The negative predictive value for iron deficiency anemia and β thalassemia trait were 83.2 % and 92.3 % respectively. Youden's index was also calculated using the formula: (Sensitivity + Specificity) - 100, which came out to be 76.9.

These values meant that the Mentzer index can be used to predict thalassemia carriers especially to remove the possibility of a diagnosis. An ideal discrimination index has high sensitivity and specificity; that is, it can detect the maximum number of patients with TT (high sensitivity) while eliminating patients with IDA (high specificity).

In this study, Mentzer Index was compared to distinguish TT from IDA by calculating their sensitivity, specificity, positive predictive value, negative predictive value, and Youden's index values. It can be concluded with this study, the cell-count-based indices, particularly the Mentzer index, are easily available and reliable methods for detecting TT.

Conclusion

β thalassemia trait and iron deficiency anemia are conditions causing microcytic hypochromic anemia. Though the definitive diagnosis depends upon iron studies and HPLC, in cases where these studies are not possible Mentzer index can be used to screen the patients. In the current study, the Mentzer index proved to be a reliable tool in differentiating between the two showing sensitivity and specificity of 87.9 % and 89 % for Iron deficiency anemia and 89 % and 87.9% for β thalassemia trait respectively. Hence, the Mentzer index can be used as a reliable diagnostic screening tool, however, confirmation by HPLC will be needed. The calculated Youden's index was significant with a value of 76.9.

What does the study add to the existing knowledge?

The HPLC is however considered to be the gold standard for diagnosing thalassemia and other hemoglobinopathies, but due to its expensiveness, it is not affordable by many peoples in developing countries like India. It is for this reason that the Mentzer index as a diagnostic screening tool was studied to differentiate between iron deficiency anemia and thalassemia trait.

Author's Contribution

Dr. Shubhi Saxena: Concept, design, literature search, data analysis, manuscript editing, manuscript review.

Dr. Richa Jain: Concept, literature search, manuscript editing, manuscript review.

Reference

- Rathod DA, Kaur A, Patel V, Patel K, Kabrawala R, Patel V, et al. Usefulness of cell counter-based parameters and formulas in detection of β -thalassemia trait in areas of high prevalence. *Am J Clin Pathol.* 2007;128(4):585-589.
doi: [Article:https://doi.org/10.1309/R1YL4B4BT2WCQDGV][Crossref]
- Lafferty JD, Crowther MA, Ali MA, Levine M. The evaluation of various mathematical RBC indices and their efficacy in discriminating between thalassemic and non-thalassemic microcytosis. *Am J Clin Pathol.* 1996;106(2):201-205.
doi: [Article:https://doi.org/10.1093/ajcp/106.2.201][Crossref]
- Sharma AK, Mehta S, Sharma S. Utility of Erythrocyte Indices for Screening of β Thalassemia Trait In Pregnant Women Attending Antenatal Clinic. *Int J Med Sci Educat.* 2016;3(4):331-337.
[Crossref]
- Sirachainan N, Iamsirirak P, Chaiyong C, Charoenkwan P, Kadegasem P, Sasanakul W et al. New Formula for the Differentiation Between Iron Deficiency Anemia and Thalassemic Carrier in Thalassemic Prevalence Areas- A Study in Healthy School-Age Children. *Blood.* 2009;114(22):1410.
doi:[Article:https://doi.org/10.1182/blood.V114.22.1410.1410][Crossref]
- Shivashankara AR, Jailkhani R, Kini A. Hemoglobinopathies in Dharwad, North Karnataka- a hospital-based study. *J Clinic Diagnos Res.* 2008;2;593-599.
[Crossref]
- Yates AM, Mortier NA, Hyde KS, Hankins JS, Ware RE. The Diagnostic Dilemma of Congenital Unstable Hemoglobinopathies. *Pediatric blood and Cancer.* 2010;55(7):1393-1395.
doi: [Article:https://doi.org/10.1002/pbc.22702][Crossref]
- Mousa AO. Types of Anemias with Low MCV Using Mentzer Index and RBC Count among Patients Seen in Basrah Al-Sadir Teaching Hospital. *Med J Babylon.* 2014;11(2):292-296.
[Crossref]
- Kementrian Keseharatan RI. Health Technology Assessment Indonesia. Pencegahan Thalassemia. 2010.
[Crossref]
- Galanello R. Prevention of Thalassaemias and other haemoglobin disorders. *Nicosia-Thalassemia International Federation.* 2003.
[Crossref]
- Patel AG, Shah AP, Sorathiya SM, Gupte SC. Hemoglobinopathies in South Gujarat population and incidence of anemia in them. *Indian J Human Genet.* 2012;18(3):294-298.
doi:[Article:https://doi.org/10.4103/0971-6866.107979][Crossref]
- Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. *Int J Lab Hematol.* 2016;38(1):32-40.
doi: [Article:https://doi.org/10.1111/ijlh.12527][Crossref]
- Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor- results in 284 patients. *Pak J Biol Sci.* 2009;12(5):473-475.
doi:[Article:http://dx.doi.org/10.3923/pjbs.2009.473.475][Crossref]
- Sirdah M, Tarazi I, Al EN, Al RH. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. *Int J Lab Hematol.* 2008;30(4):324-330.
doi:[Article:https://doi.org/10.1111/j.1751-553X.2007.00966.x][Crossref]

14. Ghafouri M, Sefat ML, Sharifi L. Comparison of cell counter indices in differentiation of beta thalassemia trait and iron deficiency anemia. *Sci J Iran Blood Transfus Organiz.* 2006;(2)385-389.
[Crossref]
15. Bain BJ. Screening of antenatal patients in a multiethnic community for thalassaemia trait. *J Clin Pathol.* 1988;41(5)481-485.
doi: [Article:<https://dx.doi.org/10.1136%2Fjcp.41.5.481>]
[Crossref]
16. Batebi A, Esmailian R. Discrimination of beta-thalassemia minor and iron deficiency anemia by screening test for red blood cell indices. *Turk J Med Sci.* 2012;42(2)275-280.
doi: [Article:<https://doi.org/10.3906/sag-0909-294>]
[Crossref]
17. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. *Pediatr Int.* 2002;44(6)612-616.
doi: [Article:<https://doi.org/10.1046/j.1442-200x.2002.01636.x>][Crossref]
18. AlFadhli SM, Al-Awadhi AM, AlKhalidi D. Validity assessment of nine discriminant functions used for the differentiation between Iron deficiency anemia and thalassemia minor. *J Trop Pediatr.* 2007;53(2)93-97.
doi: [Article:<https://doi.org/10.1093/tropej/fml070>]
[Crossref]
19. El-Agouza I1, Abu Shahla A, Sirdah M. The effect of iron deficiency anaemia on the levels of haemoglobin subtypes- possible consequences for clinical diagnosis. *Clin Lab Haematol.* 2002;24(5)285-289.
doi:[Article:<https://doi.org/10.1046/j.1365-2257.2002.00464.x>][Crossref]
20. Mentzer Jr WC. Differentiation of iron deficiency from thalassaemia trait. *The Lancet.* 1973;7808(1)882.
doi: [Article:[https://doi.org/10.1016/s0140-6736\(73\)91446-3](https://doi.org/10.1016/s0140-6736(73)91446-3)][Crossref]
21. Shine I, Lal S. A strategy to detect thalassaemia minor. *The Lancet.* 1977;8013(1)692-694.
[Crossref]
22. Schriever H, Srivastava PC. Differentiation of thalassaemia minor from iron deficiency. *The Lancet.* 1973;302(7821)154-155.
[Crossref]
23. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. *The Lancet.* 1973;7801(1)449-452.
doi: [Article:[https://doi.org/10.1016/s0140-6736\(73\)91878-3](https://doi.org/10.1016/s0140-6736(73)91878-3)][Crossref]
24. Telmissani OA, Khalil S, Roberts GT. Mean density of hemoglobin per liter of blood- a new hematologic parameter with an inherent discriminant function. *Lab Haematol.* 1999;5;149-152.
[Crossref]
25. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor. *Blood Cells.* 1989;15(3)481-495.
[Crossref]
26. Ricerca BM, Storti S, d'Onofrio G, Mancini S, Vittori M, Campisi S, et al. Differentiation of iron deficiency from thalassaemia trait- a new approach. *Haematol.* 1987;72(5)409-413.
[Crossref]