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Original Research Article

Role of Mentzer Index in Diagnostic Dilemma of Thalassemia Trait Viz A Viz Iron Deficiency Anaemia in A Teaching Hospital

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Abstract:

Background: Iron deficiency anaemia being the most common cause of anaemia in the world. The individuals suffering from thalassemia trait usually have an asymptomatic course and they present with mild microcytic hypochromic anaemia on peripheral blood smear. Since the other cause of microcytic anaemia is iron deficiency which is much more common therefore it is important to differentiate it from thalassemia trait

Objective: To determine the role of Mentzer index and Electrophoresis in differentiating between iron deficiency anaemia (IDA) and anaemia secondary to β-thalassemia trait.

Methods: This was a hospital-based retrospective observational study done on 2000 patients from December 2020 to June 2022 of all age groups. For the ease of statistical analysis patients who were found to be have iron deficiency anaemia by iron studies and thalassemia trait diagnosed by Hb electrophoresis were included in this study. History of prior blood transfusion within 3 months was excluded from this study. Mentzer index of all the patients were calculated and the results were analysed and further compared with HPLC

Results: Out of 2000 patients, 1100 patients (55%) had iron deficiency anaemia, 800 patients (40%) had β thalassaemia trait, and 100 patients (5%) had thalassaemic major. Mentzer index was found to be more reliable to detect true positive cases for β thalassemia trait with a sensitivity of 93.75%, specificity of 92.73%.

Conclusion: Iron deficiency anaemia and thalassemia with many overlapping features can be differentiated by few parameters blood indices like Mentzer index. In resource-poor and developing countries like India where socioeconomic condition is uneven, it can be used as a screening tool. Though In doubtful cases, the diagnosis can be confirmed by HPLC.

Keywords: Anaemia, β thalassemia trait, Mentzer index.

Introduction

Anaemia is a condition marked by abnormally low haemoglobin levels or when total red blood cells are lesser than the average value. Beta thalassemia trait (-TT) and iron deficiency anaemia (IDA) both cause microcytic hypochromic anaemias, which continue to be a significant burden on society, especially in the poorer developing nations. This is because IDA is much more common and B-TT is frequently mistaken for IDA because both conditions cause similar hematologic abnormalities on complete blood counts (CBCs).[1]

The most common cause of anaemia in the world is iron deficiency anaemia (IDA). [2] It is common for women to have a negative iron balance due to the loss of iron during pregnancy, delivery, and nursing. The most accurate laboratory test to distinguish IDA from anaemia caused by other illnesses is serum ferritin. [3] the most prevalent genetic illness in the world is thalassemia, which is brought on by a reduction or absence in the synthesis of one or more globin chains. According to the WHO, 5% of people worldwide are thalassemia carriers. According to research, B-thalassemia trait (BTT) is the most common single-gene disorder transmitted by heredity. [4] It is asymptomatic and frequently found during stressful times, like pregnancy. Usually, individuals with beta-thalassemia have normal iron reserves and a microcytic hypochromic blood profile. Unfortunately, they are often erroneously treated with iron. Administration of iron to these cases can be harmful and produce complications of iron overload electrophoresis. Despite Hb electrophoresis being the gold standard for thalassemia screening.[5] initial parameters routinely used are PBS, Red cell indices (MCV, MCH, MCHC), RDW due to cost effectiveness and easy availability but usually gives a diagnostic challenge to differentiate from IDA

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.[6,7]A definitive differential diagnosis between β-TT and IDA is based on the result of HbA2 electrophoresis, serum iron levels, and serum ferritin level [8] 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 anaemia.

Materials & Methods

Study Design and duration: This is a hospital based observational retrospective study done from December 2020 to June 2022 for duration of one and half year. In this study, 2000 patients of all age groups were selected. The samples were obtained and collected in EDTA anticoagulant tubes.

Thalassemia carrier diagnostic criteria were anaemia with MCV < 80fl, MCH<27pg and HbA2 fraction >3.5% [9,10,11] 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 anaemia [6,7].

The diagnosis of iron deficiency anaemia was done based on blood picture and iron studies carried out on a five-part cell differential counter and the diagnosis of β thalassemia was done based on increased HbA (>3.5%) levels estimated by BIORAD D10 Dual Reader which uses HPLC technique.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) was calculated for each measure as follows:

- Sensitivity = [True positive (True positive + False negative)] × 100
- Specificity = [True negative (True negative + False positive)] × 100
- PPV = True positive (True positive + False positive) × 100
- NPV = True negative (True negative + False negative) × 100

Inclusion criteria

1. All Patients already being diagnosed with iron deficiency anaemia or thalassemia trait based on blood picture, iron studies, and HPLC.
2. Patients of all age groups.

Exclusion Criteria

1. Coexistence of other haematological conditions like autoimmune haemolytic anaemia, aplastic anaemia presence of β thalassemia trait along with iron deficiency anaemia in the same patient
2. History of blood transfusion in near were excluded from this study

Operational Definitions

Anaemia

Haemoglobin of less than 10.0 g/dL at any time during pregnancy in developing countries, according to WHO.

Mentzer Index

The Mentzer index was first described by Mentzer in the year 1973. It is calculated from the CBC report. Mentzer index (MCV/RBC count) of less than 13 may represent thalassemia trait, and greater than 13 often indicates IDA[6,7].

HPLC

During screening for classical beta-thalassaemia trait, the hallmark is the presence of an elevated level of HbA2 [12] this necessitates the accurate estimation of Hb A2 (α2δ2).

HPLC is a sensitive and precise method for the identification of Hb A2, Hb F and abnormal haemoglobins. An automatic HPLC system, the VARIANT (Bio-Rad, 2000) [13,14] is currently available primarily for the detection of β-thalassaemia carriers and the common abnormal haemoglobins (Hb S, Hb C, HbE). In this study, we used the Variant, HPLC system to confirm the findings of red cell indices.

This VARIANT (Bio-Rad, 2000), is a fully automated high performance liquid chromatography (HPLC) system which uses double-wave length detection (416 and 690 nM). This cationic-exchange column chromatography enables qualitative determinations of Hb A2, Hb F and abnormal haemoglobins in 6.5 minutes on a haemolysate prepared from 5 ml of venous blood.

Results

Peripheral blood analysis of 2000 patients included in this study showed microcytotic hypochromic anemia. Blood samples run through a 5-part cell counter showed decreased Hb, PCV, MCV, MCH, MCHC. Iron deficiency anaemia was confirmed by conduct of iron studies to exclude other differential causes.

Thalassaemic trait was diagnosed based on elevated HbA2 as measured by HPLC. A total of 2000 patients enrolled in this study were analysed. Of these, 1100 patients (55%) had iron deficiency anaemia, 800 patients (40%) had β thalassaemia trait, and 100 patients (5%) had thalassaemic major. (fig1)

Mentzer index was calculated, among 1100 cases of IDA 180 cases, the index value was <13 and 920 cases value were >13, among 800 cases of β

thalassemia trait, 650 cases the index value was <13 and 150 cases the value was >13 (table 1)

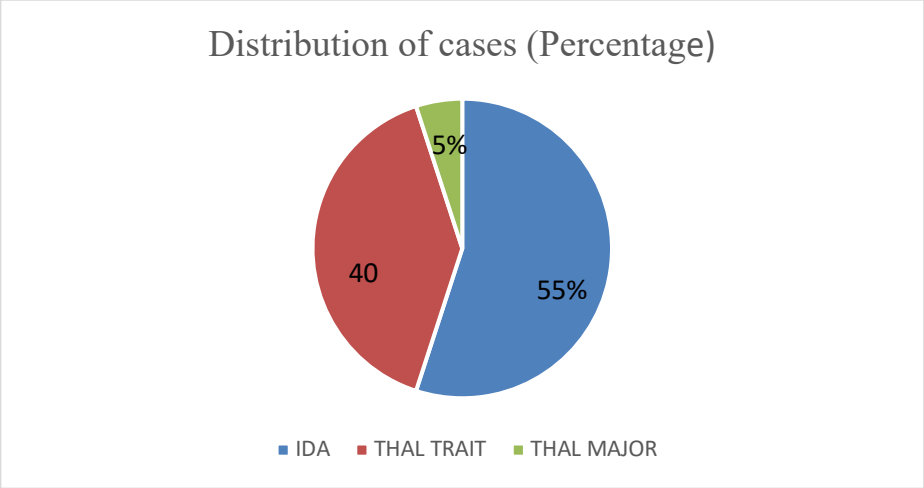

Figure 1: Total number of patients afflicted

Table 1: Mentzer index among IDA and Thalassemia Trait

Cases	Mentzer index <13	Mentzer Index >13
IDA	180	920
THAL TRAIT	650	150

An evaluation of the clinical presentation of patients was conducted. The most frequent clinical characteristic observed in patients with iron deficiency was anemia, with fatigue being the most common symptom. Other common symptoms included breathlessness, irritation, anorexia, and fatigue. In comparison, the majority of patients with β thalassaemia trait presented with symptoms of asymptotia, although some of them also exhibited fatigue and irritability, as well as breathlessness and anorexia.

The formulae used to calculate the sensitivity and specificity of each patient, as well as the negative predictive value and Youden's index, were outlined in the relevant materials and methodologies. 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 93.75 % whereas it was more specific to pick the true negative cases of Iron Deficiency anaemia with a specificity of 93.75 %. The positive predictive values for iron deficiency anaemia and β thalassemia trait were 95.30 % and 90.30 % respectively. The negative predictive value for iron deficiency anaemia and β thalassemia trait was 90.30 % and 95.30 % respectively. Youden's index was also calculated using the formula: (Sensitivity + Specificity) – 100, which came out to be 86.48. (fig 2, table 2)

Figure 2: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Youden's Index of iron deficiency anaemia and β thalassemia trait

Table 2: Sensitivity, specificity, PPV, NPV of IDA vs Thalassemia Trait

	Iron Deficiency Anaemia	Thalassemia Trait
Sensitivity	92.73%	93.75%
Specificity	93.75%	92.73%
Positive Predictive Value	95.30%	90.30%
Negative Predictive value	90.30%	95.30%
Accuracy	93.16%	0%

Discussion

The World Health Organization (WHO) has reported that a fifth of the world's population is affected by thalassaemia, a genetic disorder caused by a deficiency or absence of the production of a certain type of globin. Iron deficiency and thalassaemic trait are two of the most frequent causes of Microcytic Anemia in India. It is important to distinguish between these two conditions as they have distinct causes, prognoses, and treatments. Children are more likely to suffer from iron deficiency due to dietary deficiencies, growth suppression, and the presence of helminths. Thalassaemia is a major disease burden in South-East Asia, particularly in India, where the population is disproportionately affected by the condition due to the higher number of patients with thalassic trait[15]

If a diagnosis of β thalassemia trait is not made, it can lead to the development of potentially homozygous offspring with the potential to develop β thalassaemia disease. The symptoms of patients with both iron deficiency anaemia (IDA) and β thalassemia trait are typically similar, with the majority of them being asymptomatic. These symptoms typically include tiredness, shortness of breath, weight loss, abdominal anorexia, facial stomatitis and irritability. The majority of patients with β thalassic trait remain symptomatic and develop symptoms in their later years. Iron deficiency anaemia is diagnosed based on a decrease in peripheral blood vessel count (PCV), central blood vessel count (MCV), major blood vessel count (MCH) and major blood vessel function (MCHC) but is confirmed by conducting iron studies. Common findings of iron deficiency include a decrease in serum ferritin, a decrease in serum iron, and an increase in total iron binding capacity.

Thalassemia is diagnosed based on the presence of elevated levels of haemoglobin A2 (HbA2) in the blood (>3.5%) on a high-throughput linear accelerator (HPLC) andmutation analysis [16]The Mentzer index (mean corpuscular volume/red blood cell count ratio) was used by Mentzer WC et al from Turkey to distinguish between the thalassemia trait and iron deficiency anemia in 290 children aged 1 to 16 years. These findings showed that the Mentzer index, which had a sensitivity of 98.7% and a specificity of 82.3%, was the most accurate predictor. In 2009, Ehsani et al. demonstrated that the Mentzer index (90.1%) was the best discrimination index per Youden's standards, followed by the Ehsani et al. index (85.5%). Mentzer and Ehsani et al. were successful in correctly diagnosing 94.7% and 92.9% of cases in their study, respectively[17].Similar results were found by Batebi et al who reported 86.3% and 85.4% for the sensitivity and specificity of the Mentzer index[18].

More than 200 mutations have recently been discovered in the -globin gene. Different gene mutations will result in varying degrees of clinical signs and symptoms in individuals, as well as variations in blood indices including Hb, RBC, and MCV levels. Lower Hb, RBC, and MCV values will result from B+ mutations in globin chains than from + mutations. Although iron studies and HPLC are the conclusive tests for iron deficiency anemia and thalassemia, respectively, it is challenging to perform these tests in all patients with microcytic hypochromic anemia due to their high cost. However, the HbA2 analysis is regarded as the gold standard for thalassemia diagnosis. Red cell indices have been calculated using electronic cell counters as the initial TT indication.

By utilizing indices to differentiate across anemia types, researchers can identify patients who are likely to need the right kind of follow-up care and cut down on wasteful costs associated with useless research. Since 1970, several complete blood count indices have been put out as easy-to-use, low-cost techniques for determining whether a blood sample is more suggestive of TT or IDA. Due to this, the Mentzer index was investigated as a diagnostic screening tool to distinguish between iron deficiency anemia and thalassemia trait. Mentzer index is a calculation of the MCV/RBC ratio in which patients with a value of 13 are identified as thalassemia carriers and those with a value of 13 are identified as iron deficient.

The diagnosis ofB 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 . 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 cellcount-based indices, particularly the Mentzer index, are easily available and reliable methods for detecting Thalassemia Trait.

Conclusion

Iron deficiency anaemia and thalassemia have different effects on blood indices. β thalassemia trait and iron deficiency anaemia are conditions causing microcytic hypochromic anaemia. Iron overload is a common complication among thalassemia patients, leading to much serious co-morbidity. Not only transfusion-dependent thalassemia patients suffer from this condition, it can also occur in NTDT patients who have not been transfused due to an increased intestinal absorption of iron. Iron supplements could be harmful to these patients. The Mentzer index can be used as a primary tool in low resource settings. They can be used as screening to select patients for Hb electrophoresis (gold standard) and prevent unnecessary parenteral iron supplementation to the cases of BTT.

Strength of this study: Despite the fact that thalassemia is common in north eastern parts of India, not many studies were conducted on incorporating the routine use of Mentzer index in cases of microcytic hypochromic anaemia used primarily to differentiate among IDA and thalassemia trait. The usual diagnosis involves use of HPLC incurring high cost as a result many cases remain undiagnosed and untreated.

Limitation of this study: Lack of proper follow up and all doubtful cases were not being corroborated with HPLC findings.

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