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RESEARCH ARTICLE

CORRELATION BETWEEN RBC INDICES, MENTZER INDEX AND HPLC FINDINGS IN PATIENTS OF ANEMIA

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ABSTRACT

Background: Anemia is a commonly encountered health problem in India. Beta thalassemia and iron deficiency anemia (IDA) are one of the important causes of microcytic hypochromic anaemia. Their differentiation is important because they not only require different management but also have different implications to the community. High Performance Liquid Chromatography (HPLC) is currently the gold standard in diagnosing haemoglobinopathies. However, in a resource poor country like India HPLC may not be widely available, especially in rural areas. Mentzer's index is commonly used to differentiate IDA and thalassemia. The objective of this study is to correlate the HPLC findings with the red cell indices and Mentzer's index and study its sensitivity and specificity in differentiating IDA from thalassemia trait. **Methods:** A cross sectional study was done on 500 anemic patients in a tertiary care centre in Western Maharashtra. Complete blood counts, HPLC was done on patients' blood samples and Mentzer's index was calculated. Statistical analysis of data was done by SPSS 16 software. **Results:** The sensitivity and specificity of Mentzer's index in differentiating thalassemia from IDA is 39.06% and 79.86% respectively. **Conclusion:** Mentzer index is not a reliable marker in diagnosis of thalassemia as it has low sensitivity and specificity. RBC indices have a better correlation and may be used as surrogate markers for the diagnosis of IDA along with serum iron studies in cases where HPLC is not available.

INTRODUCTION

Anemia is a commonly encountered condition in India, wherein there is a decrease in the oxygen carrying capacity of the blood. Anemia can be due to many causes of which iron deficiency anemia and haemoglobinopathies, notably thalassemia are most common. Iron deficiency anemia is an easily treatable condition usually requiring oral iron supplements or in severe cases parenteral iron therapy or blood transfusions. Beta Thalassemia and Iron Deficiency Anemia are the important causes of microcytic hypochromic anaemia and their differentiation is important because they not only require different management and treatment but also have different implications to the patient's family, community or society. Beta Thalassemia requires pre-marriage counselling while iron deficiency anemia needs assessment of nutritional status. Whereas thalassemia and thalassemia hemoglobinopathies are genetic disorders requiring lifelong blood transfusion support along with iron chelation therapy. Thalassemia is an autosomal recessive inherited group of disorders of hemoglobin synthesis characterized by the absence or reduction of one or more of the globin chains of hemoglobin. The structural variants result from substitution of one or more amino acids in the globin chains of the hemoglobin molecule (Weatherall and Clegg, 2001).

The only curative treatment of thalassemia and other haemoglobinopathies, is a HLA matched hematopoietic stem cell transplant. Plethora of hemoglobin variants is prevalent in India owing to ethnic diversity of its population with minimal to major clinical significance. These diseases pose a serious health problems leading to severe morbidity and mortality in Indian population. It is therefore important to correctly diagnose the cause of anemia as a correct diagnosis will help prevent the unnecessary iron loading of a thalassemia patient. Differentiating iron deficiency anemia from thalassemia carrier status is a frequently faced challenge in medical practice, in particular in subjects with mild or moderate iron deficiency anemia and in regions where thalassemia is common. It is not possible to distinguish both conditions using simple routine blood counts, as they are both associated with microcytic and hypochromic erythrocytes. High Performance Liquid Chromatography (HPLC) is currently considered the gold standard in diagnosing haemoglobinopathies. However in a resource poor country like India HPLC may not be widely available especially in rural areas. This has led to increase in financial burden involving serum iron studies and haemoglobin electrophoresis along with genetic counselling. Various studies have proposed different indices in different populations for early differentiation of these two conditions depending on

RBC parameters which are easily available on automated cell counters. We intend to correlate easily available parameters like Hemoglobin (Hb), RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW) with HPLC findings. Mentzer's index is defined as mean corpuscular volume per red cell count.

$$\text{Mentzer Index} = \frac{\text{MCV (in femtolites per cell)}}{\text{RBC count in millions}}$$

An index of less than 13 suggests that the patient has thalassemia trait whereas greater than 13 suggests iron deficiency anaemia. We will also study the sensitivity and specificity of Mentzer's index in differentiating iron deficiency anemia from thalassemia trait.

Review of literature

In a study conducted by Aysel Vehapoglu *et al.* in School of Medicine, Istanbul, Turkey, they retrospectively evaluated the reliability of various indices such as red blood cell (RBC) count; red blood distribution width index; the Mentzer, Shine and Lal, England and Fraser, Srivastava and Bevington, Green and King, Ricerca, Sirdah, and Ehsani indices; mean density of hemoglobin/liter of blood; and mean cell density of haemoglobin for differential diagnosis of microcytosis and β -Thalassemia trait in the same patient groups. Mentzer index was found to be the most reliable index in differentiating β -Thalassemia trait from Iron deficiency anemia (Aysel Vehapoglu *et al.*, 2014). Articles from India and Turkey describe similar diagnostic challenges and possible approaches for more cost-effective screening of iron deficiency anaemia. The first study, was done by Sazawal *et al.* in Delhi. This study used two haematological indices, namely haemoglobin concentration ≤ 10 g/dL and red cell distribution width (RDW) $>15\%$, to identify iron-deficient children between 1 and 3 years of age (confirmed by zinc protoporphyrin and serum ferritin assays). Statistical analysis confirmed a sensitivity of 99% and specificity of 90% if haemoglobin and RDW alone were used for screening (Sazawal *et al.*, 2014). The second study (Mentzer, 1973), 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 betathalassaemia trait from iron deficiency anaemia. These results indicated that the Mentzer index was the most reliable indicator, with a sensitivity of 98.7% and specificity of 82.3% (Mentzer, 1973). In a study conducted by Lawrie D; Glencross, D K. in South African children, they tried to study the use of Mentzer's index in early detection of iron deficiency anemia. They concluded that cell-count-based indices, particularly the Mentzer index, are easily available and reliable methods for detecting β -Thalassemia Trait. According to their results, the percentage of correctly diagnosed patients was the highest with the Mentzer index (91%) followed by the Ehsani *et al.* index (84.8%). The third highest one was RBC count (83.4%). Cell-count-based parameters and formulas, particularly the MCV and RBC counts and their related indices (Mentzer index and Ehsani *et al.* index), were found to be good discrimination ability in diagnosing β -Thalassemia Trait (Lawrie and Glencross, 2015). In a study conducted by Soontharee Plengsuree *et al* in Thai adult population with the aim of examining the diagnostic accuracy of five red cell indices [red blood cell (RBC) counts, mean corpuscular

volume (MCV), mean corpuscular hemoglobin (Hb) (MCH), mean corpuscular Hb concentration (MCHC), and red cell distr

ibution width (RDW)] and nine formulas (RDW/RBC, RDW Index, Sirdah, Green and King, Mentzer, England and Fraser, Ehsani, Srivastava and Shine and Lal). Their sensitivity, specificity, positive predictive value (PPV), and negative predictive values (NPV), efficiency, and Youden's Index were analyzed in 102 β -thal trait and 64 iron deficiency adult Thai subjects. The RDW/RBC formula proved to be the most reliable index as they had 100.0% specificity and PPV and the highest efficiency (94.58%) and Youden's Index (91.18%), as well as high sensitivity (91.18%) and NPV (87.67%). Therefore, they found that this formula could be used in initial discrimination of β -thal trait from iron deficiency in adult Thai subjects (Soontharee Plengsuree *et al.*, 2015). In a study conducted by Johannes J.M.L. Hoffmann, Eloisa Urrechaga, and Urko Aguirre, they carried out a meta analysis of 12 indices developed by various researchers for distinguishing iron deficiency anemia from thalassemia including Mentzer's index, Ehsani's index, Shrivastava's index, M/H ratio, etc. Their meta-analysis has demonstrated high variation in the performance of discriminant indices for distinguishing thalassemia trait from iron deficiency anemia. In general, the newer indices seem to be able to make this distinction better than the more traditional formulas. They have also shown that age (adult or child) and geographical region, but not the type of hematology analyzer, are important factors determining the diagnostic utility of the discriminant indices (Johannes *et al.*, 2015).

Aims and objective

1. To determine hemoglobin and other red cell indices on samples collected from patients with clinical symptoms of anemia.
2. To carry out HPLC on the above samples and correlate the HPLC findings with the red cell indices.
3. To calculate the Mentzer's index and study its sensitivity and specificity in differentiating iron deficiency anemia from thalassemia trait.

MATERIALS AND METHODS

Type of study: Cross Sectional Observational study.

Study population: Anemic patients attending OPD at Command Hospital (Southern Command) or getting admitted to Command Hospital (Southern Command) and referred to Dept. of IH & BT, AFMC for HPLC.

Sample size: A sample size of 500 was taken over a period of four months based on our work load and as estimated from previous studies.

Inclusion criteria: All patients who were found to have haemoglobin count low for their age/sex as per the WHO criteria for anemia.

Children (6-59 months): less than 11.0 gm%.
 Children (5-11 years): less than 11.5 gm%.
 Children (12-14 years): less than 12 gm%.
 Non-pregnant women: less than 12 gm%.
 (15 yrs and above)

Pregnant women: less than 11gm%
Men: less than 13 gm%.

Exclusion criteria: All patients having hemoglobin count greater than the cutoff values as per the WHO guidelines (mentioned above).

Methods used: Informed consent was taken from patients who are referred to the department of IH and BT, AFMC for HPLC. They will be interviewed and their details will be recorded. 3 ml blood samples will be collected in EDTA vacutainers. Hemoglobin and Red cell Indices will be measured using Automated Hematology analyzer (Sysmex). HPLC was carried out on Bio-Rad D-10 hemoglobin testing System as per the manufacturer's standard operating instructions. Patient data was collected from the blood bank of AFMC as per the laid down procedure and tabulated in an excel sheet. Statistical analysis was done using appropriate statistical tests using SPSS 17 software in consultation with a biostatistician.

Observations: A total of 500 samples were collected for carrying out HPLC out of which 437 were found to have anemia as per WHO criteria on carrying out haemoglobin and Red Cell Indices measurement using Sysmex and were included in the study. The remaining 63 samples were excluded from the study (Table 1).

Table 1. Sample size studied

Samples Studied	500
Anaemic (who criteria)	437 (Included)
a. Thal trait	45
c. Other haemoglobinopathies	
i)HBE heterozygous	8
ii)HBE homozygous	7
iii)Sickle cell heterozygous	3
iv)Iron deficiency anemia	374
NON – ANEMIC	63 (EXCLUDED)

1. Comparison of RBC Indices in Thalassemic and Iron deficiency anemia

In my study, the mean RBC indices in Thalassemic patients v/s patients with iron deficiency anemia are as shown in the table 2. It is seen from the review of literature that the normal RBC indices are:-

RBC count >6.1million/cumm; MCV, 80 femtolitre/cell; MCHC, 33 grams/decilitre and MCH, 27 picogram/cell. In our study, the RBC indices varied significantly in patients of Thalassemia compared to patients of Iron deficiency anemia as shown in Table 3. Abnormal haemoglobin indices (RBC count > 6.1 million/cumm; MCV <80 femtolitre /cell; MCHC < 33 g/dl and MCH < 27 picogram/ cell) was found in 23.8 % of Thalassemic patients compared to 5.6% in non Thalassemic patients.

2. Comparison of Mentzer Index in Thalassemic and Non Thalassemic patients (Table 4)

Validity of Mentzers Index in differentiating Thalassemia from Iron deficiency anemia

1. Sensitivity = 39.06%
2. Specificity = 79.89%

Mentzers index below 13 was found in 39.06% of thalassemic patients compared to 20.1% in non thalassemic patients. As mentioned in Table 4

Correlation coefficient between mentzer index and HbA2 = - 0.117 (not significant).

Table 2. Mean RBC indices in Thalassemic patients v/s patients with iron deficiency anemia.

Mean values	Thalassemia	Iron deficiency anemia	P value
RBC	5.23	4.56	1.0002
MCV	72.18	76.39	.0022
MCH	20.78	22.05	0.134
MCHC	29.35	28.25	0.007

Table 3. Comparison of blood indices in patients of Thalassemia and iron deficiency anemia

Blood indices	Thalassemia		Total
	Positive	Negative	
Abnormal	15	21	36
Normal	48	353	401
Total	63	374	437

P value = 0.0013 (Fischer test)

Table 4. Comparison of Mentzer Index in Thalassemic and Non Thalassemic patients

Mentzer Index (Cutoff 13)	Thalassemia		TOTAL
	Present	Absent	
Less than 13	25	75	100
More than 13	39	298	337
Total	64	373	437

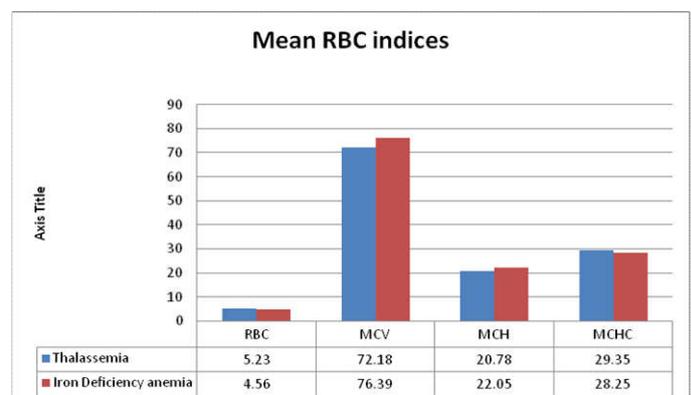


Fig 1. Comparison of RBC Indices in Thalassemi A and Iron deficiency anemia

RESULTS

The sensitivity and specificity of Mentzers index in differentiating Thalassemia from iron deficiency anemia is 39.06% and 79.86% respectively. Abnormal Mentzer index (less than 13) was found in 38.46% of Thalassemic patients compared to 20.34% of non thalassemic patients. Abnormal haemoglobin indices (RBC count > 6.1 million/cumm; MCV <80 femtolitre /cell; MCHC < 33 g/dl and MCH < 27 picogram/ cell) was found in 23.8 % of Thalassemic patients compared to 5.6% in non Thalassemic patients.

DISCUSSION

Differentiating iron deficiency anemia from thalassemia carrier status is a frequent issue in medical practice, in particular in subjects with mild or moderate iron deficiency anemia and in

regions where thalassemia is common. It is not possible to distinguish both conditions using simple routine blood counts, as they are both associated with microcytic and hypochromic erythrocytes. However, in thalassemia RBC do tend to be more microcytic, whereas iron deficient RBC are often more hypochromic (Lafferty *et al.*, 1996; d'Onofrio *et al.*, 1992). These differences have been exploited by developing simple mathematical formulas for emphasizing the differences in RBC indices as a tool for distinguishing iron deficiency anemia from thalassemia trait (Aysel Vehapoglu *et al.*, 2014; Sazawal *et al.*, 2014; Mentzer, 1973; Lawrie *et al.*, 2015; Soontharee Plengsuree *et al.*, 2015; Johannes *et al.*, 2015). However, the discriminative power of these simple indices never reached maximum diagnostic performance. The large number of discriminant indices described in the literature reflects that researchers were continuously stimulated devising new and supposedly better indices for applying in their local patient population. In the last decade, multiple studies have been published which compared different discriminant indices in the same patient cohort, aimed at identifying the index with the best overall performance. However, none of them proved to be completely adequate for differentiation and more over one index may show greater sensitivity and specificity in one population but proved to be ineffective in another population (Sazawal *et al.*, 2014; Lawrie *et al.*, 2015). Vehapoglu *et al.*, have concluded in their study from Turkey that Mentzer index has the highest reliability for differentiating Beta Thalassemia and iron deficiency anemia while Shen *et al.*, concluded the superiority of Green and King (GK) index, Ricerca (Ric) index and England and Fraser (EF) index in Chinese children (Matos *et al.*, 2013). In contrast, in Brazilian and Palestinian population GK index RDWI and Sirdah (Si) Index have proved to be effective for differentiation (Shen *et al.*, 2010). The Differences in the effectiveness of various discriminating indices in different (Chandra *et al.*, 2016) populations may be attributed to mutational spectrum of thalassaemia in varied regional areas with each mutation affecting a certain amount of globin chain synthesis and making changes in indices accordingly (Chandra *et al.*, 2016). A metaanalysis was carried out by Johannes J.M.L. Hoffmann, EloísaUrrechaga, and Urko Aguirre in order to find the discriminant indices with the highest overall performance. It was the first time that this subject was investigated using a meta-analysis. They found that most importantly, the designs of the studies investigated were far from homogeneous: there was huge variation in patient selection criteria, in types of thalassemia included, in geographical origin of the patients, in type of hematology analyzer used and in cut-off value for the respective discriminant indices. As each of these factors may play a role in the diagnostic utility of the index (Johannes *et al.*, 2015). In this study, we analysed Mentzer Index, however the utility of the same was found to be questionable.

Conclusion

So, from this study we can conclude that there is no direct correlation between Mentzer index and HbA2 findings in patients of anemia. Mentzer index is a not a reliable marker in diagnosis of thalassemia since, it has got very low sensitivity and specificity and has higher incidence of false positive and false negatives. RBC indices give a better correlation with the diagnosis of Thalassemia and Iron deficiency anemia and may be used as a surrogate marker for the diagnosis of Iron deficiency anemia along with serum iron studies in cases where HPLC is not available. HPLC remains to be the gold

standard in the diagnosis of haemoglobinopathies. Assessing HbA2 values is the most reliable method in the diagnosis of Thalassemia.

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