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RESEARCH ARTICLE

EVALUATION OF THE DIAGNOSTIC IMPORTANCE OF MENTZER INDEX FOR SCREENING THALASSEMIA MINOR AND DIFFERENTIATING IT FROM IRON DEFICIENCY

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Abstract: Background and Aim: Mentzer index can differentiate microcytosis due to Thalassemia from other causes in a minimal resource setting and therefore, it can be playing a significant role in screening of Thalassemia in general population as well as distinguishing β Thalassemia trait from iron deficiency anemia has important clinical implications. The misdiagnosis of β Thalassemia trait has consequences for potential homozygous offspring leading to β Thalassemia disease. We aim to evaluate the diagnostic importance of Mentzer Index for screening Thalassemia minor as compared to other indices and differentiating it from other causes of microcytic anemia at our center. **Materials and Methods**: This is a retrospective analytical study done over a period of 2 years from 1, Jan 2021 to 30, Dec 2022. In this study, a retrospective analysis of all the patients whose High-Performance Liquid Chromatography (HPLC) was performed at our laboratory and had microcytic hypochromic RBC picture, was done. Mentzer Index of all these patients was calculated and the results were analyzed and further compared with HPLC. The cut off for Mentzer Index was taken as 13. **Results**: Total patients screened during the study were 176, out of which 54 patients were Thalassemia minor diagnosed through HPLC. Sensitivity of Mentzer index to detect thalassemic patient was 79.4%, specificity 95.3%, positive predictive value 90% and negative predictive value was found to be 89.7%. Sensitivity of Mentzer index is higher when compared to other indices in our study. **Conclusion**: Mentzer Index can be calculated from Complete blood counts with red blood cell indices can be used to target population who should be screened for Thalassemia even in a resource poor setting. Various studies and now ours proves Mentzer index to be a good and cheaper alternative to screen patients at primary health centers and can help to reduce the burden of Thalassemia major in society.

KEYWORDS: Mentzer Index, Thalassemia, High-Performance Liquid Chromatography, microcytosis

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INTRODUCTION:

Microcytosis is a common red cell change seen in anemias of varied etiology. These include iron deficiency, Thalassemia, anemia of chronic disease and sideroblastic anemia's. [1,31] With the use of Red blood cell indices and Mentzer index we can differentiate microcytosis due to Thalassemia from other causes.[2] This approach can help clinicians to screen Thalassemia in general population at primary healthcare level with minimal resources and can play an important role in control of Thalassemia in our country. The most common cause of anemia worldwide is iron deficiency anemia,[31] which can be suspected from a low MCV and presence of hypochromic, microcytic red cells. Many studies has shown the importance of Red cell indices derived from automated red cell analyzers which have been used to differentiate between patients with microcytic red blood cells with a high probability of Thalassemia minor from others.

Most common cause of microcytic hypochromic anemia in our developing nation is Iron deficiency anemia. Laboratory confirmation of iron deficiency can be measured by iron profile and staining of bone marrow aspirates for iron. [3] Microcytosis seen in Thalassemia minor cases are usually associated with increased red blood cell count and normal or near normal hemoglobin despite reduction of mean volume (MCV) and mean cell hemoglobin (MCH). In contrast, iron deficiency anemia MCV and MCH do not fall until the hemoglobin is significantly reduced.[4] Although, the definite differential diagnosis between β-Thalassemia trait and Iron deficiency anemia is based on the measurement of HbA2 and HbF by electrophoresis or genetic analysis,[5] but with help of these parameters we can screen patients at primary level. Patients with microcytic hypochromic anemia having MCV <80 fl and HbA2 fraction >3.5% were considered positive for Thalassemia trait. [6]

This being a preventable condition, it is all the more important to identify, educate and counsel the carriers to lower the incidence of this life-threatening condition. Our way of achieving this goal is to screen the population at risk at primary level itself which can play an important role in control of Thalassemia in our country. [7]

MATERIALS AND METHODS:

This is a Retrospective study done over a period of 2 year from Jan 2021 to Dec 2022 where all patients who came for HPLC with history of microcytic hypochromic anemia were screened. Mentzer index of all patients with microcytosis was calculated. The results were analyzed and further compared with HPLC. Mentzer Index is calculated from red cell indices (mean corpuscular volume, Total red cell count) using a formula of Mentzer Index = (MCV/red blood cell count). Cut off for Mentzer Index was taken as 13. Confirmatory test was done by Bio-Rad D-10, an automated cation –exchange HPLC (High Performance Liquid Chromatography Instrument Hemoglobin) instrument that has been used to quantify Hb A2, Hb F, Hb A along with screening other hemoglobin variants. All patients with MCV<80fl were included. The cut off limit for Hb A2 in Thalassemia minor patients was taken as >3.5 and statistics were applied as and when required. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each measure as following formulas:

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

The ethical approval for this study and the need for informed consent was not needed in our study.

Statistical Analysis: Appropriate statistical methods have been applied wherever necessary and results have been expressed as percentage, charts, tables and diagrams.

RESULTS:

During the study 1112 patients were who came for HPLC over the period of two years were screened for microcytosis which was found in 176 patients. Complete blood counts with red cell indices were done in these patients by an automatic five-part analyzer XN550 which works on a principle of impedance technology. All the patients with MCV< 80fl, MCH<25 pg were diagnosed as having Microcytic hypochromic blood picture. Further, Mentzer index of 176 patients were calculated as defined above and patients were divided into two categories [**Table 1**], the first being mentzer index <13, these were assumed to be having thalassemic microcytosis due to Thalassemia trait mostly and the second category mentzer index >13 were diagnosed as having non thalassemic microcytosis which were further screened for iron status. HPLC was done and analysed and patients were divided on the basis of HbA2 levels. The patients with HbA2 >3.5 were confirmed to be having Thalassemia trait.

Table 1: comparison of mi index between thalassemic patients and non-thalassemic patients

CATAGORY	MI INDEX >13	MI INDEX <13
THALASSAEMIC	14	40
NON THALASSAEMIC	114	8

The parameters analyzed and compared for all the patients were age, sex, mean hemoglobin levels, mean MCV, mean red blood cell counts for both thalassemic and non-thalassemic patients. [**Table 2 and 3**]

Table 2: Age group of all patients screened and for thalassemia minor patients

Age Group	NO OF PATIENTS (No)	%	AGE GROUP FOR THALASSEMIC MINOR PATIENTS	NO OF PATIENTS	%
0-9	8	4.5	0-9	4	7.1
10-19	10	5.7	10-19	4	7.1
20-29	38	21.6	20-29	10	17.8
30-39	70	39.8	30-39	18	32.1
40-49	20	11.4	40-49	6	10.7
50-59	22	12.5	50-59	8	14.3
>60	14	7.9	>60	8	14.3

Table 3: comparison of parameters among thalassemic patients and non-thalassemic patients

PARAMETER	HB	MCV	Red cell counts
AVAERAGE FOR THAL MINOR	9.95	63.44	5.1
AVAERAGE FOR NON THALASEMIC	8.8	70.4	4.2

We performed validity tests (sensitivity, specific, Negative Predictive Value, Positive Predictive Value) **[Table 4]** to assess the diagnostic value of Mentzer index to screen for Thalassemia trait in subjects as below:

Sensitivity = [54/ (54 + 14)] × 100= 79.4%

Specificity = [122/ (122 + 6)] × 100=95%

Negative Predictive Value = 122/ (122 + 14) × 100=89.7%

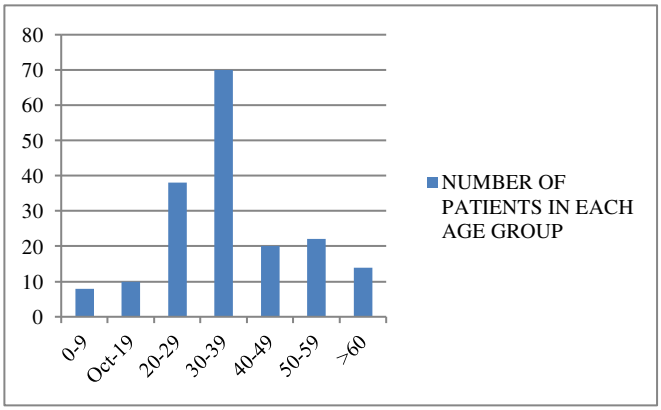
Positive Predictive Value = 54/(54+6) × 100= 90%

Table 4. Positives and negatives

	POSITIVE	NEGATIVE
POSITIVE	**TRUE POSITIVE (54)**	**FALSE POSITIVE (6)**
NEGATIVE	**FALSE NEGATIVE (14)**	**TRUENEGATIVE (122)**

For Thalassemia trait the most commonly screened patients were from age group 30-39 years with total of 39.8%[**Figure.1**], which were mostly females of child bearing age and screening was done during antenatal checkup, this was followed by 20-29 years age group which were 21.6% again mostly females. We saw a trend that males were less likely to be screened.

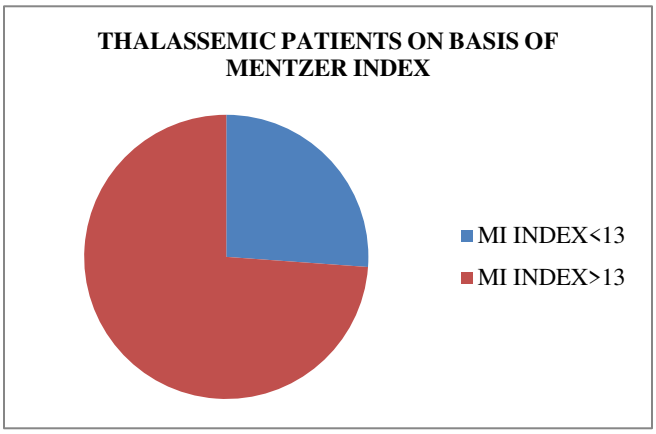
Figure.1 Showing age distribution of patients screened for thalassemia



On comparing the mean hemoglobin levels among the two groups it was found that mean Hb for thalassemic patients was 9.95gm/dl and mean MCV was 63.44 fl and mean RBC count was 5.1 million. For non-thalassemic group the mean hemoglobin level was 8.8gm/dl which is slightly lower, mean MCV was 70.4fl and mean RBC count was 4.2 million.

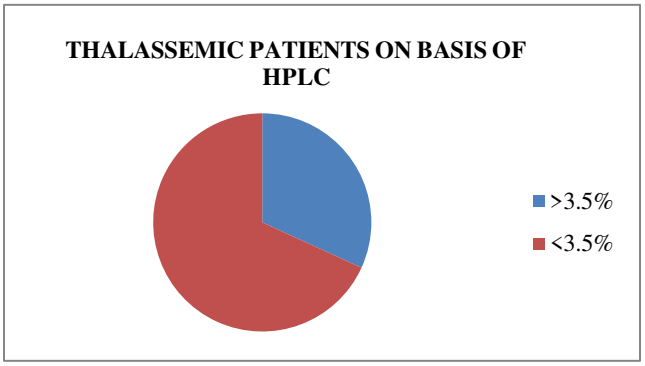
On comparing the Mentzer Index of 176 patients [**Figure. 2**] it was found that 46 patients (prevalence of 26.1%) were having MI index <13 and 130 patients (73.8%) were having Mentzer index >13.

Figure. 2 Number of thalassemic patients on basis of Mentzer index



On comparing the HPLC analysis of these patients it was found that out of 176 patients 54 patients were confirmed to be having Thalassemia trait. [**Figure 3**] And for all the patient's serum iron and ferritin levels were recorded from EMR were ever available.

Figure. 3 number of thalassemic patients on basis of HPLC



On comparing both the results it was found that with the help of Mentzer index we were able to correctly identify 40 patients out of 54 confirmed and 14 patients were false positive. Iron study was also analyzed for all the cases were ever available. In these 14 patients 4 patients were having HbE variant hemoglobin, 8 patients (14.3%) were having concurrent iron deficiency anemia as the serum iron

levels were below the normal limits. For remaining 2 cases no cause identified.

We also calculated sensitivity of different other index's and found that Mentzer index has higher sensitivity as compared to others. [**Table 5**]

Table. 5 Comparison of sensitivity of Mentzer index with other indices

S.NO.	INDICES	FORMULA	SENSITIVITY
1	MENTZER INDEX	MCV/RBC	79.8
2	RDWI	MCV*RDW/RBC	65.8
3	SRIVASTAVA INDEX	MCH/RBC	75
4	SIRDAH	MCV-RBC-(3*HB)	60
5	EHSANI	MCV-(10*RBC)	77.1
6	RICERCA INDEX	RDW/RBC	55.1
7	MDHL	(MCH/MCV) *RBC	67.5
8	MCHD	MCH/MCV	51.9
9	GREEN AND KING	(MCV*MCV*RDW)/ HB*100	64.3

DISCUSSION:

Differentiating iron deficiency anemia from Thalassemia trait has an important clinical implication. Therefore, a reliable diagnosis is needed in order to reduce unnecessary laboratory testing and avoid in appropriate treatment. The clinical presentation of iron deficiency anemia and Thalassemia trait are usually similar and consists of pallor, easy fatiquability and breathlessness, anorexia, pica and angular stomatitis.[27] The diagnosis of iron deficiency anemia depends upon reduced PCV, MCV, MCH, and MCHC along with iron profile. G Ajeet et al. [8] A wide range of parameters are available to facilitate this differentiation between iron deficiency and Thalassemia trait. However, no single parameter alone or in any combination was able to discriminate. [6]

Our aim is to reintroduce an already existing simple, doable and affordable screening tool to identify patients with Thalassemia carrier genes at primary healthcare level with minimal facilities.

In our study a total of 1112 patients were screened, out which we found that 176 patients are having microcytic hypochromic blood picture, taking consideration of our cut off values decided on the basis of various earlier studies like by A Sufia et al. [9] And out of these total 1112 patients 54(4.6%) patients were having Thalassemia trait. The prevalence of Thalassemia trait in Rathod DA et. al. was about 3.3% close to our study. [10] Study by T Arulmani et al. tells that National carrier state of Thalassemia is 5% to 7%. [11] The most common age group having Thalassemia trait found in our study was 30-39yrs with 18 patients (32.1%) followed by age group 20-29yrs with 10 patients (17.8%), the average age of patients was 25 years in study conducted by Majida et al. [12] The least common age group to be screened for Thalassemia were children's and elderly. The prevalence may vary and could be higher because of selective screening as seen in our study, most patients screened are predominantly females during their antenatal checkups, in general Thalassemia remains undetected because of less awareness among people which can lead to increase of burden of Thalassemia major in our society.

Thalassemia syndromes are among the most common genetic disorders worldwide, with 1.7% of the world's population carrying thalassemic genes as stated by U Eloísa et al. [13] India has higher incidence of Thalassemia, consanguineous marriages in some parts of India could be the contributing factor.[11]

Various studies show different indices and formulas which are being used to screen and differentiate Thalassemia trait and iron deficiency anemia anemia as stated by V aysel et al and others. [14-21] [**Table 6**].

Table. 6 Comparison of our study with others

PARAMETER	sensitivity	Specificity	PPV	NPV
OUR STUDY	79.4	95	89.7	90
Saxena S. et al	89.0	87.9	83.2	92.3
V Aysel et al [k]	98.7	82.3	Not calculated	Not calculated

When compared with other Mentzer index has proved to reliable in many studies also seen in study by Lafferty JD et al. [22,23]. Our study also shows that Mentzer index has better sensitivity then other indices hence is a better screening tool for thalassemia trait. [**Table 5**]

The cutoff of mentzer index was taken as 13 in our study, similar cut off was taken by G Ajeet et al. [24]

True Positives, False Positives, Positive Predictive Value and Negative Predictive Value were calculated from data. [**Table 4**]. Sensitivity and specificity of mentzer index as per our study is 79.8% and 95% which is close to study done by Saxena S. et al [25] Sensitivity of Mentzer index is higher followed by EHSANI (77.1%) and SRIVASTAVA INDEX (75%) in our study. However, there are other studies also available showing more sensitivity of other index. [26, 27] While out of 56 patients with Mentzer index less than 13, 6 patients (10.7%) had Mentzer index less than 13 and HbA2 less the 3.5. Amita et al. [28] Iron deficiency and thalassemia can be seen in same patient also mentioned in study by sarika V et al. [29,30]

CONCLUSION:

Thalassemia diagnosis requires hemoglobin (Hb) electrophoresis using electrophoresis or High-Performance Liquid Chromatography (HPLC) device or gene examination using Polymerase Chain Reaction (PCR) device which is not available at primary health centers.

Mentzer Index can be calculated from Complete blood counts with red blood cell indices which is a most commonly ordered test and can be used to target population who should be screened for Thalassemia even in a resource poor setting.

Various studies and now ours proves that Mentzer index is one of the good and cheaper alternatives to screen patients at primary health centers and can help to reduce the burden of Thalassemia major in society. Red blood cell indices, and the various formulas derived from red blood cell parameters, have been proposed as simple, rapid and inexpensive test methods that can be initially used to distinguish Thalassemia trait.

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