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Role of Mentzer Index for Differential Diagnosis of Iron Deficiency Anaemia and Beta Thalassemia Trait

Tej Prakash Shah,[1] Anjan Shrestha,[2] Jagdish Prasad Agrawal,[1] Suman Rimal,[1] Anita Basnet[3]

[1]Depatment of Medicine, Tribhuvan University Teaching Hospital, [2]Department of Pathology, Tribhuvan University Teaching Hospital, [3]Patan Academy of Health Sciences.

ABSTRACT

Background: The most common differential diagnoses of microcytic hypochromic anaemia are iron deficiency anaemia and beta thalassemia. Globally, thalassemia affects approximately 4.4 out of every 10,000 live births whereas iron deficiency anaemia comprises half of all anaemia worldwide as per world health organization. The definitive diagnosis of beta thalassemia trait and iron deficiency anaemia requires haemoglobin analysis and iron studies respectively, which are not possible to perform in all suspected cases especially in resource limited settings. The study aims to evaluate the reliability of mentzer index in differentiating beta thalassemia trait from iron deficiency anaemia.

Methods: This was a cross sectional, observational study done on 59 patients each of beta thalassemia trait and iron deficiency anaemia from August 2019 to July 2020. Patients who were found to be having iron deficiency anaemia diagnosed by iron studies and beta thalassemia trait diagnosed by Hb electrophoresis were enrolled in the study using simple random sampling technique.

Results: Mentzer index correctly identified 95.76% of overall patients. Area under receiver operating characteristic curve was 0.993 (95% CI, 0.985-1.002, $p<0.001$). For beta thalassemia trait, mentzer index showed a sensitivity of 93.2%, specificity of 98.3%, positive predictive value of 98.2%, negative predictive value of 93.5%; while for iron deficiency anaemia, sensitivity of 98.3%, specificity of 93.2%, positive predictive value of 93.5% and negative predictive value of 98.2%. Youden's index was 91.5.

Conclusions: The findings of the present study make mentzer index a reliable screening method, especially in a resource poor setting, like Nepal. Further confirmation by gold standard tests is recommended.

Keywords: Beta thalassemia trait; iron deficiency anaemia; mentzer index

INTRODUCTION

Iron deficiency anaemia (IDA) and Beta thalassemia trait (BTT) are the most common causes of microcytic hypochromic anaemia.[1,2] IDA mimics BTT in red blood cell (RBC) indices whereas causes, treatment and prognosis are different.[3] Correct diagnosis is important to decide whether to supplement or prevent iron supplementation and to prevent lethal forms of thalassemia by premarital counselling.[1,4]

Gold standard tests for IDA and BTT are time consuming and expensive, thus require complementary methods in resource limited settings for screening.[1,4] Mentzer index/MI (MCV/RBC) of more than 13 indicates IDA, while MI of less than 13 indicates BTT. The study aimed to find the role of MI in differentiating BTT and IDA. The study also aimed to compare the demographic profile, hematological and biochemical parameters of both diseases as well as to find a correlation of MI with serum ferritin and transferrin saturation (TS) in IDA and with HbA2 in BTT patients.

METHODS

This was a cross-sectional quantitative study conducted in Tribhuvan University Teaching Hospital (TUTH). The patients attending Hematology Outpatient Department (OPD) and patients admitted in Medicine Ward were enrolled in the study from August 2019 to July 2020. Prior to the data collection, ethical approval was obtained from the Institutional Review Committee, Institute of Medicine. Prior written informed consent was taken from the patients or patients' relative or caregiver wherever appropriate. Participants who were 16 years and above and diagnosed with IDA or BTT on the basis of blood picture, iron studies and capillary electrophoresis were included in study. Patients with other hematological

Correspondence: Tej Prakash Shah, Depatment of Medicine, Tribhuvan University Teaching Hospital, Maharajgunj, Kathmandu, Nepal. Email: rauniyar.tejprakash@gmail.com, Phone: +9779840058319.

morbidities, haemoglobinopathies, blood transfusion history within past 3 months and pregnant women were not included in the study. Sample size (SS) was calculated using $SS = Z^2 \times P \times (1 - P) / C^2$, where Z is Z value, P is prevalence and C is margin of error. Since prevalence studies of BTT have not been done in Nepal, we assumed a prevalence of 4 % (similar to India), with a Z value of 1.96 for 95 % confidence interval, and margin of error of 5 %. Using this formula, 59 patients of BTT and a equal number of IDA patients for comparison were selected using simple random sampling technique. Microcytic anaemia was defined as per the standard case definition of anaemia from WHO (Hb < 13g/dl in men, < 12 g/dl in non-pregnant women) with microcytic hypochromic picture on peripheral blood smear (PBS) and mean cell volme (MCV) < 80 fl.[5] IDA was diagnosed in cases with a low serum ferritin (≤ 12ng/ml) and a TS < 16%. BTT was diagnosed in cases with HbA2 > 3.5% on capillary electrophoresis, serum ferritin > 12ng/ml and TS > 16%.[6-8] The data were analyzed using computerized statistical package for social sciences (SPSS) version 20. An independent sample □-test was performed to detect differences between the two groups. Nominal categorical data such as gender was analyzed with chi square test or fisher's exact test with consideration of p-values < 0.05 as significant. The predictive ability of MI was tested by plotting the area under receiver operating characteristic (ROC) curve. Correlation of MI with HbA2 in BTT and with TS and ferritin in IDA patients was determined using Pearson correlation analysis.

RESULTS

A total of 59 cases were of confirmed IDA with serum ferritin levels ≤ 12 ng/dl, TS < 16% whereas 59 cases of BTT with HbA2 > 3.5%, serum ferritin > 12ng/dl and TS > 16%. The mean age (in years) of IDA participants was 44.8 ± 17 and that of BTT was 30.7 ± 12.9. Tharu and janajati comprised the major chunk of participants with BTT, forming 30.5% and 22% respectively. 80% of the BTT patients had family history.

Table 1. Hematological and biochemical data of study groups.

	BTT (n : 59)		IDA (n : 59)	
	Range	Mean ± SD	Range	Mean ± SD
Hb (g/dl)	8-12.6	10.5±1.2	5.5-12	8.8± 1.6
MCV (fl)	46-78.90	59.39± 5.87	55-79	73.75± 5.24
RBC (millions/µl)	4.1-8.13	5.7±0.8	2.04-5.3	3.6± 0.7
SI (µg/dl)	49-161	94.3± 28.3	4.8-35	22.1± 7.4
Ferritin (ng/ml)	15.7-457	111.3± 92.9	1.1-11.9	7.6± 2.9
TIBC (µg/dl)	191-399	307.8± 55.9	354-593	432.3± 49.5
TS (%)	23.13-49.21	30.29± 5.63	0.94-9.89	5.3± 2.09

There were significant differences between BTT and IDA group in terms of hematological and biochemical parameters. In this study, Hb (g/dl) values in the BTT group were 10.5 ± 1.2, and those in the IDA group were 8.8 ± 1.6, the difference was significant with p < 0.001. MCV (fl) was 59.39 ± 5.87 in the BTT group, lower than those in the IDA group (73.75 ± 5.24; p < 0.001). RBC count (millions/µl) was higher in the BTT (5.7 ± 0.8) group than that in the IDA group (3.6 ± 0.7; p < 0.001). Serum iron/SI (µg/dl) was higher in the BTT group (94.3 ± 28.3) than that in the IDA group (22.1 ± 7.4; p<0.001). Serum ferritin (ng/ml) was lower in the IDA group (7.6 ± 2.9) compared to BTT group (111.3 ± 92.9; p < 0.001). Total iron binding capacity/TIBC (µg/dl) in the IDA group was higher (432.3 ± 49.5) than the BTT group (307.8±55.9; p < 0.001). TS (%) in the BTT group was 30.29 ± 5.63, and that in the IDA group was 5.3 ± 2.09, p < 0.001 (Table 1).

Table 2. Mean Mentzer index in BTT and IDA patients.

Diagnosis	BTT (n : 59)		IDA (n : 59)	
	Range	Mean ± SD	Range	Mean ± SD
Mentzer index	6.77 - 16.05	10.61 ± 2.01	12.83 - 34.51	21.18 ± 4.72

In comparison of mentzer index between IDA and BTT group, a significant difference between IDA and BTT score was observed with mean value of 21.18 ± 4.72 and 10.61 ± 2.01 respectively with statistical association (p-value < 0.001) (Table 2).

Table 3.Diagnostic accuracy of Mentzer index.

Mentzer index	BTT (n:59)	IDA (n:59)	Correctly diagnosed %
>13	4	58	95.76
<13	55	1	

Using Mentzer index, 55 out of 59 BTT patients were correctly diagnosed, with 4 patients having MI >13. 58 patients of IDA were correctly diagnosed and only 1 patient had MI < 13. Overall, 95.76 % of patients were correctly diagnosed using mentzer index with a cut off value of 13 (Table 3).

Table 4. ROC analysis of predictive reliability of Mentzer index in diagnosing IDA and BTT.

Test Result Variable	Area	Std. Error	P-value	95% Confidence interval	
				Lower Bound	Upper Bound
Mentzer index	0.993	0.004	<0.001	0.985	1.002

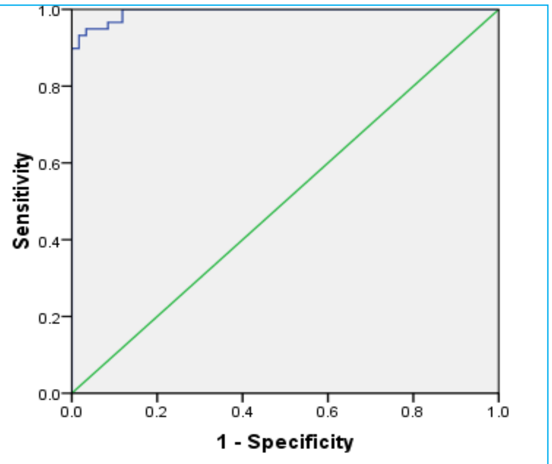

Figure 1. ROC curve of Mentzer index for its predictive value in diagnosing IDA and BTT.

Area under ROC curve for MI with a cut off value of 13 in predicting a correct diagnosis of BTT or IDA with 95% CI was 0.993 [0.985 – 1.002]. The finding was significant with a p value < 0.001 (Table 4, Figure 1).

Table 5. Sensitivity, specificity, PPV, NPV, Youden's index of Mentzer index

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's Index
BTT	93.2	98.3	98.2	93.5	91.5
IDA	98.3	93.2	93.5	98.2	

For BTT, MI showed a sensitivity of 93.2%, specificity of 98.3%, positive predictive value (PPV) of 98.2%, negative predictive value (NPV) of 93.5%, and for IDA, sensitivity of 98.3%, specificity of 93.2%, PPV of 93.5% and NPV of 98.2% respectively. Youden's index, calculated as (sensitivity + specificity) - 100, was 91.5 (Table 5).

Table 6. Pearson correlation analysis of Mentzer index with TS and Ferritin in IDA and with HbA2 in BTT

Diagnosis	Pearson correlation (r)		
	HbA2	TS	Ferritin
BTT (n:59)	-0.295 (p=0.023)	–	–
IDA (n:59)	–	-0.403 (p=0.002)	-0.274 (p=0.036)

Using pearson correlation analysis, MI showed a weak negative correlation with HbA2 in BTT patients and also with TS and ferritin in IDA patients (Table 6).

DISCUSSION

In present study, MCV (fl) was 59.39 ± 5.87 in the BTT group, lower than that in the IDA group (73.75 ± 5.24). The difference in between the two groups was comparable with p < 0.001. RBC count (millions/µl) was higher in the BTT (5.7 ± 0.8) group than that in the IDA (3.6±0.7; p < 0.001) group. These findings are consistent with previous studies that compared different discriminant functions in IDA and BTT patients, which showed that BTT patients had lower MCV values than IDA patients and higher RBC counts compared to IDA patients. In the study by Ehsani et al, MCV of BTT patients was 62.04 ± 4.57, and that of IDA patients was 70.04 ± 7.97; RBC count in the BTT group was 5.89 ± 0.59 and that in the IDA group was 4.41 ± 0.55.[2] Likewise, in the study conducted by Sirdah et al, MCV in the BTT group was 66.44 ± 5.29, and in the IDA group, it was 72.57 ± 5.83; RBC count in the BTT group was 5.93 ± 0.57, and 4.92 ± 0.61 in the IDA group.[9]

In the present study, mentzer index correctly identified 55 out of 59 BTT patients and 58 out of 59 patients of IDA. Overall, 95.76% of patients were correctly diagnosed using MI with a cut off value of 13. Accuracy of MI of 95.76 % in present study is greater than that of Kumar A (89.6 %) and Vehapoglu (91%), and lower than that of Ehsani (97.71%).[2, 8, 10]

In the present study, area under ROC curve was 0.993 (95% CI 0.985-1.002, p < 0.001), indicating a high predictive reliability of MI in differentiating BTT from IDA. For BTT, MI showed a sensitivity of 93.2%, specificity of 98.3%, PPV of 98.2%, NPV of 93.5%, and for IDA, sensitivity of 98.3%, specificity of 93.2%, PPV of 93.5% and NPV of 98.2%. Youden's index was 91.5. Youden's index in the studies conducted by Kumar A, Ehsani , Shreya B, Vehapoglu and Okan V were 69, 90.1, 75, 81 and 60 respectively.[2,8,10,11,12] Youden's index in the present study is the highest compared to other studies, validating the reliability of MI in differentiating between BTT and IDA in Nepalese population. However, the inclusion of patients with lower Hb values compared to other studies might limit the applicability of findings of this study in those patients who have Hb just below normal range.

In present study, MI showed a weak negative correlation with HbA2 in BTT patients (inverse correlation; r: -0.295, p = 0.023), indicating that a higher HbA2 level was associated with a lower MI value. In IDA patients, it showed a weak negative correlation with TS (r: -0.403, p = 0.002) and a weak negative correlation with ferritin (r: -0.274, p = 0.036). In the study conducted by Amid A et al, spearman correlation analysis showed that MI correlated with HbA2 level in BTT patients (inverse correlation; ρ: -0.23, p < 0.001); ferritin level was not correlated with MI in iron deficient patients (ρ: 0.043, p=0.718).[13] We could not find studies which performed a correlation analysis of MI with TS.

The study was conducted in a single center. So, findings of the study couldn't be generalized in all settings. Further, lower Hb values in the IDA group as compared to BTT group might be a limiting factor for a useful comparison of MI in differentiating between these two entities. Further, Hb analysis results (by capillary electrophoresis) were obtained from only 25 out of 59 IDA patients, so the study might have falsely included IDA patients with concomitant BTT. Moreover, DNA studies for mutation analysis were not performed.

CONCLUSIONS

The study finds the prevalence of BTT was highest in the Tharus. There were significant differences between MCV and RBC counts in between BTT and IDA groups. Mentzer index showed a weak negative correlation with HbA2 in BTT patients, and weak negative correlations with TS and ferritin in IDA group. In our study population, MI with a cut off value of 13 proved to be reliable in differentiating BTT from IDA, with an accuracy of 95.76 %, area under ROC curve of 0.993% and youden's index of 91.5. MI can be used as a reliable screening tool for differentiating BTT from IDA. Further confirmation by gold standard tests such as iron studies for IDA and Hb analysis and DNA mutation analysis for BTT is recommended.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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