

Diagnostic Potential of Haematological Indices (Mentzer Index and RDWI) to Distinguish Iron Deficiency Anemia and β TT

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ABSTRACT

Background: Iron Deficiency Anemia (IDA) and β -thalassemia trait (β TT) are the most frequently encountered entities that present with mild microcytic hypochromic anemia. Distinguishing between IDA and β TT is essential to circumvent unnecessary iron therapy and make an incorrect diagnosis of β TT. The high cost and inaccessibility of specific confirmatory tests have led to the utilization of different red blood cell (RBC) indices for differentiation between IDA and β TT. RDWI and Mentzer Index (MI) are cost-effective, easily available and valid screening tools for screening and identification of IDA and β TT. The objective of this study was to assess the diagnostic accuracy of RDWI and Mentzer Index for screening and differentiation of IDA and β TT traits in terms of cost-effectiveness, availability to be used as a valid screening tool in identifying patients with a high index of suspicion in resource constraint settings.

Patients and methods: This analytical study was conducted at Hematology, Pathology Department, Service Institute of Medical Sciences/Services Hospital, Lahore, from March to September 2021. A total of 322 patients presenting with microcytic hypochromic anemia (Hb, HCT, MCV, MCH, MCHC, all reduced on CBC and confirmed on peripheral smear morphology) were enrolled after taking informed consent. IDA was diagnosed on the basis of low serum ferritin (<15ng/ml) and β TT by HPLC, showing HbA2 >3.5%. CBC parameters were obtained by an automated hematology analyzer. Serum Ferritin was measured by ELISA using reagent kits. HPLC was performed on Biorad D-10. The Mentzer index and RDWI were calculated from parameters obtained by the automated analyzer. All the findings were recorded in proforma. The MI <13 and RDWI <220 favors β TT. The MI >13 and RDWI >220 favors IDA. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy and positive likelihood ratio for Mentzer and RDWI were calculated for IDA and β TT. Data was entered and analyzed in SPSS 21.

Results: The mean age of the cases in IDA group was 16.7 years and in β TT it was 22.4 years. There were 101 (44.5%) males and 126 (55.5%) females in IDA; and there were 32 (33.7%) males and 63 (66.3%) females in the β TT groups. The MI has a PPV of 88.6%, a NPV of 64.6%, an accuracy of 79.9%, and a positive likelihood ratio of 3.47. Serum ferritin was a reference to diagnose IDA. However, it is not cost-effective, and MI, as a screening tool with a high PPV, accuracy and positive likelihood ratio of 3.47, is likely to detect IDA 3.47 times more than serum ferritin. RDWI has a positive predictive value of 93.8%, a negative predictive value of 74.8%, an accuracy of 87.2%, and a positive likelihood ratio of 6.44 for IDA. Thus, it is 6.44 times likely to detect IDA than serum ferritin.

Conclusion: The MI and RDWI are valid screening tools with high diagnostic accuracy for initial screening and differentiation of IDA and β TT in resource-constraint settings. Haematological indices, particularly RDWI, are effective in identifying microcytic hypochromic anemia and distinguishing it from β TT.

Keywords:

Iron Deficiency Anemia, β -thalassemia trait, Red Cell Distribution Width Index, Mentzer Index, Diagnosis

INTRODUCTION

Thalassemia is a set of genetic blood disorders that affects people all over the world. β -thalassemia trait (β TT) is believed to afflict 3% of the population, affecting 60,000 children every year, with Asian nations accounting for 80% of these cases. In Pakistan, 5000

cases of thalassemia are detected each year. The carrier rate in our nation ranges from 5% to 8%.¹ A study in 2012 indicated the prevalence of β TT to be 6.5% in the general Quetta population.² According to EMRO WHO statistics, it has been found that more than one-fifth of women in Pakistan suffer from anemia.³ Recent statistics show a prevalence of around 45% of Iron deficiency anemia in Pakistan, which is extremely high reflecting the failure of public health measures in controlling it.⁴ IDA and β TT are both characterized by microcytosis and hypochromia. IDA mimics β TT in CBC parameters but etiology, treatment and prognosis

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is different. Correct diagnosis is essential to decide whether or not to supplement iron and prevent Thalassemia major by premarital counselling.⁵ Gold Standard test (Iron Studies and HPLC) for IDA and β TT are quite expensive and available only at specialized centres, thus it mandates use of complementary methods in resource limited settings for screening.⁶ Various studies worldwide and in Pakistan have proposed discriminating indices to differentiate the two entities in low-resource settings. Mentzer, RDWI, Shine and Lal, RDW, Green and King, England and Fraser, RBC are a few amongst other indices proposed.^{5,6} Cross-sectional study conducted at Hematology unit of Hayatabad Medical complex reported sensitivity and specificity of Mentzer index of 74.81% and 70.06%. Positive predictive value and negative predictive values were 78.94% and 64.96%, respectively. The diagnostic accuracy of Mentzer index was 72.91% and thus concluded it to be a beneficial discriminant tool through a cost effective way.⁷ Another Study in India concluded Mentzer index 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 TT respectively.⁸ Study by Biva Rani Mondal et al reported that RDWI had both sensitivity and specificity more than 80% in detection of BTT and IDA.¹⁰

Using RBC indices to differentiate between IDA and β TT has the potential to identify high-risk people for follow-up and reduce needless investigations.⁹ The distinction between IDA and β TT is highly significant, thus if β TT is mistaken as IDA, Hb will not improve, and the physician may prescribe extra iron.¹¹

Keeping in mind the high prevalence of IDA and β TT in our population, it is of utmost importance to develop a single cost-effective test which can filter the cases of these two mimicking conditions. Various studies have concluded RDWI and Mentzer to be more sensitive, specific and accurate to discriminate β TT and IDA, thus the present study aimed to determine the diagnostic accuracy of RDWI and Mentzer Index for Screening and Differentiation of IDA and β TT.

PATIENTS AND METHODS

This was an analytical study which was conducted in the Department of Hematology, Services Institute of Medical Sciences, Lahore, from March 2021 to September 2021. Patients coming to hematology department for evaluation of anemia, of both genders and age 1-65 years presenting with microcytic hypochromic anemia (Hb, HCT, MCV, MCH, MCHC

all reduced on CBC and confirmed on peripheral smear morphology) were included. Patients already taking hematinics, diagnosed with hematological disease other than thalassemia and history of transfusion within previous 1 month were excluded from the study.

A definitive diagnosis of IDA and β TT is based on the results of serum ferritin and hemoglobin quantification/separation by HPLC, respectively. IDA was diagnosed on basis of low serum ferritin (<15ng/ml). β TT was diagnosed by HPLC showing HbA2 >3.5%. The detail of the study was explained to respondents fulfilling the inclusion criterion. Demographic details age, gender, weight were recorded. After informed consent, 5ml peripheral venous blood was collected following aseptic measures in EDTA tube and another 2ml venous blood collected in tube without anti-coagulant for serum separation. RBC count, Hb, MCV, MCH, MCHC, HCT, RDW were obtained by Hematology Analyzer (Sysmex Automated Hematology Analyzer). Peripheral smear of the patients was made and reviewed for morphology. Mentzer Index and RDWI were calculated from parameters obtained by automated CBC analyzer. The Mentzer index was calculated as the MCV/RBC ratio. RDWI was calculated as MCV x RDW/ RBC. Serum Ferritin was measured by ELISA using reagent kits. HbA, HbA2, HbF were studied by High performance liquid chromatography method (Biorad-D10). All the findings were recorded in proforma. Mentzer index <13 and RDWI < 220 favors BTT. Mentzer Index >13 and RDWI >220 favours IDA.

Collected data was then edited and analyzed according to the objectives by IBM software statistical package for social science (SPSS 22). Ethical clearance was taken from IRB of the institution. Sensitivity, Specificity, PPV, NPV, Accuracy and likelihood ratio of Mentzer Index and RDWI for IDA and BTT was calculated and analyzed. Screening test are considered effective when sensitivity and specificity is between 80-90% and positive likelihood ratio 4-9.

$$\text{Sensitivity} = [\text{TP} / (\text{TP} + \text{FN})] \times 100$$

$$\text{Specificity} = [\text{TN} / (\text{TN} + \text{FP})] \times 100$$

$$\text{PPV} = [\text{TP} / (\text{TP} + \text{FP})] \times 100$$

$$\text{NPV} = [\text{TN} / (\text{TN} + \text{FN})] \times 100$$

$$\text{Accuracy} = [(\text{TN} + \text{TP}) / (\text{TP} + \text{FP} + \text{TN} + \text{FN})]$$

$$\text{Positive Likelihood Ratio} = \text{sensitivity} / (100 - \text{specificity})$$

RESULTS

Total no of patients meeting inclusion criterion were 322. Among them 227 cases were identified as Iron Deficiency Anemia and 95 as Beta Thalassemia trait. There were 101 (44.5%) males and 126 (55.5%) females

Table 1: Demographics and CBC parameters

Characteristics	IDA (227)	β eta-Thalassemia Trait (95)	p-value
Age (Mean \pm S.D)	16.78 \pm 12.39	22.46 \pm 12.80	0.001
Gender			
Male	101 (44.5%)	32 (33.7%)	0.072
Female	126 (55.5%)	63 (66.3%)	
Weight (Mean \pm S.D)	41.86 \pm 22.54	54.54 \pm 22.07	< 0.001
Hb (Mean \pm S.D)	8.05 \pm 2.01	9.76 \pm 2.23	< 0.001
MCV (Mean \pm S.D)	64.25 \pm 8.82	61.10 \pm 6.47	0.002
MCH (Mean \pm S.D)	18.79 \pm 4.34	18.35 \pm 3.22	0.379
MCHC (Mean \pm S.D)	28.58 \pm 4.45	30.33 \pm 3.20	0.001
RDW (Mean \pm S.D)	19.96 \pm 4.35	16.85 \pm 4.10	< 0.001
RBC	4.24 \pm 0.81	5.34 \pm 0.93	<0.001
HCT	27.40 \pm 5.82	32.37 \pm 6.29	< 0.001
Mentzer	15.86 \pm 4.73	11.77 \pm 2.85	< 0.001
RDWI	314.49 \pm 117.57	197.86 \pm 64.64	< 0.001

Table 2: Hemoglobin analysis by HPLC

Hb type	IDA (227) Mean \pm S.D	β -Thalassemia Trait (95) Mean \pm S.D	p-value
HbA	96.67 \pm 0.72	93.47 \pm 1.48	<0.001
HbA2	2.45 \pm 0.44	5.29 \pm 0.96	<0.001
HbF	0.77 \pm 0.52	1.30 \pm 1.34	<0.001

with IDA. Similarly, there were 32 (33.7%) males and 63 (66.3%) females with β TT.

The mean of RBC, HCT, Mentzer and RDWI was 4.24 \pm 0.81, 27.40 \pm 5.82, 15.86 \pm 4.73 and 314.49 \pm 117.57 in IDA, respectively. Similarly, the mean of RBC, HCT, Mentzer and RDWI was 5.34 \pm 0.93, 32.37 \pm 6.29, 11.77 \pm 2.85, and 197.86 \pm 64.64 respectively in β TT (Table 1).

Demographic details and other mean of other CBC parameters are shown in Table 1. The mean of HbA, HbA2, and HbF was 96.67 \pm 0.72, 2.45 \pm 0.44, and 0.77 \pm 0.52 in the IDA, respectively. Similarly, the mean HbA, HbA2, and HbF were 93.47 \pm 1.48, 5.29 \pm 0.96, 1.30 \pm 1.34, respectively, in the β TT (Table 2). The sensitivity and specificity of Mentzer in IDA was 81% and 76.6% while in β TT it was 76.6% and 81% RDWI showed sensitivity and specificity 87.6% and 86% in IDA and 86% and 87% in β TT. The positive predictive value, Negative Predictive Value and Positive likelihood ratio of Mentzer and RDWI for IDA and β TT are shown in Table 3.

DISCUSSION

IDA and β TT are the leading causes of microcytic anaemia. Distinguishing between these two entities is essential to circumvent unnecessary iron therapy and incorrect diagnosis of β eta-thalassemia trait.¹¹ Adoption of national thalassemia prevention programs which focus on educating masses, carrier screening is ideal and more suitable in regions of high prevalence and limited resources.¹² To decrease the cost, and to overcome unavailability of specialized tests at most places,

numerous RBC indices and formulae have been applied to distinguish IDA and β TT. The majority of β TT patients are asymptomatic, and without specialized testing, they may go undetected or be misdiagnosed as IDA.

In this study out of 322 subjects, 227 (70%) patients were diagnosed to be having IDA and 95 (30%) were labeled as having β TT. The mean age of the cases in iron deficiency anemia was 16.78 \pm 12.39 and in β TT it was 22.46 \pm 12.80. Similarly, in a previous study the mean age in IDA was 28.86 \pm 14.58 and in β TT was 21.98 \pm 16.37.¹³ There were 101 (44.5%) male and 126 (55.5%) females in IDA; there were 32 (33.7%) males and 63 (66.3%) females with β TT. The mean Hb in patients with IDA was 8.05 \pm 2.01 and those with β TT was 9.76 \pm 2.23. In a previous study the mean Hb was close to our findings 10.60 \pm 1.21 in IDA and 10.95 \pm 1.20 in β TT.¹² Mean MCV was 64.25 \pm 8.82 in IDA and 61.10 \pm 6.47 in β TT. In a previous study mean MCV value was 71.87 \pm 6.93 in IDA and 62.17 \pm 4.14 in β TT.¹³ In this study the mean RBC was 4.24 \pm 0.81 in IDA and 5.34 \pm 0.93 in β TT. A previous study showed mean RBC in IDA 4.8 \pm 0.7 and in β TT 5.28 \pm 0.63.¹² The findings of the current study showed mean HCT 27.40 \pm 5.82 in IDA while 32.37 \pm 6.29 in β TT. Similar results were found in previous research with mean HCT 32.7 \pm 3.71 in IDA and 33.31 \pm 4.09 in β TT.¹² In this study, the mean of Mentzer index was 15.86 \pm 4.73 in IDA and 11.77 \pm 2.85 in β TT. Similarly, Idrees et al. (2023) reported in their results the mean of the Mentzer index was 17.39 \pm 7.48 in IDA and 13.16 \pm 7.27 in β TT.¹⁴ In this study, RDWI showed sensitivity and specificity of 87% and 86% in IDA and 86% and 87%, respectively, in β TT. Jameel and researchers reported that RDWI has a sensitivity of 80.7% and a specificity of 84.7 for β TT, while sensitivity and specificity for IDA are 84.7% and 80.5%, respectively.¹⁵ The sensitivity and specificity of Mentzer in IDA group was 81% and 76% while in β TT it was 63% and 96%. Our results are similar to the results reported by Ashok et al with sensitivity and specificity of 76% and 81%, respectively.¹⁶ Another study computed the sensitivity of Mentzer index for β TT to be 68%.¹⁷ Mentzer Index has positive predictive value 88.6%, negative predictive value 64.6%, accuracy 79.9%, positive likelihood ratio of 3.47. MI as a screening tool with high positive predictive value, accuracy and positive likelihood ratio of 3.47 is likely to detect IDA 3.47 times more than serum ferritin. Study by Atika sherali et al has reported positive likelihood ratio of 3.6 of Mentzer index for iron deficiency anaemia.⁹

Table 3: Sensitivity, specificity, PPV, NPV of MI, and RDWI

	Sensitivity	Specificity	PPV	NPV	Accuracy	PLR
Mentzer Index (MI)						
IDA	81.4	76.6	88.6%	64.6%	79.9%	3.47
β Thalassemia Trait	76.6%	81.4%	64.6%	88.6%	79.9	4.11
RDWI						
IDA	87.6%	86.4%	93.8%	74.8%	87.2%	6.44
β Thalassemia Trait	86.4%	87.6%	74.8%	93.8%	87.3%	6.96

RDWI has positive predictive value 93.8%, negative predictive value 74.8%, accuracy 87.2%, positive likelihood ratio of 6.44 for IDA, thus it 6.44 times likely to detect IDA than serum ferritin. These findings are similar to that reported by Biva rani mondal.¹⁰ Similar results were obtained for Mentzer and RDWI for IDA and βTT.

CONCLUSION

This study concludes that the Mentzer index and RDWI serve as valid screening tools for initial screening and differentiation of IDA and βTT. They are readily available and cost-effective, in identifying patients with high index of suspicion who require appropriate follow-up with specialized confirmatory tests.

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