DOI: 10.7860/JCDR/2016/17672.7843

Original Article

Pathology Section

Evaluation of Platelet and Red Blood Cell Parameters with Proposal of Modified Score as Discriminating Guide for Iron Deficiency Anemia and β-Thalassemia Minor

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ABSTRACT

Introduction: Iron Deficiency Anaemia (IDA) and β-Thalassaemia Minor (BTM) are considered to be important cause of microcytic hypochromic anaemia. Studies have evaluated various red cell parameters which are easily available on electronic cell counters for discrimination of IDA and BTM in different ethnic populations. The analysis of previously established red cell discriminative indices with new cut-off have also been done by studies which may be relevant in their set of population for differentiation.

Aim: The study was conducted to propose a modified score considering the established red blood cell indices with a new cut off and to formulate index taking into consideration Red Blood Cell (RBC) and platelet parameters for early differentiation of IDA and BTM.

Materials and Methods: The prospective study included cases with MCV< 80 fl and new modified score of 11 was proposed by statistically analysing the previous discriminative indices with new cut-off by giving score 0 for IDA and score 1 for BTM. The summation of all scores gave modified 11 T score. A new cut off for differentiation of IDA and BTM was proposed in the study by using ROC curve and analysing AUC which statistically corresponded to highest accuracy. An attempt to formulate a new index using the RBC and platelet parameters was also made for initial discrimination.

Results: The study included 153 cases and in addition to red blood cell parameters, mean platelet volume and platelet distribution width also showed statistical significant difference between IDA and BTM ($p<0.05$). Modified new 11 T score was 87.6% specific for BTM while proposed index showed 80.4% negative predictive value for BTM and correctly identified 75% of cases.

Conclusion: The proposed new index and modified 11T score may be used for initial discrimination of BTM and IDA especially in resource limited regions. Apart from RBC parameters, mean platelet volume and platelet distribution width may also be useful in early differentiation. It is essential to determine cut off of every index in given population for differentiation of these two conditions.

Keywords: Hematological indicators, Microcytic hypochromic anemia, Platelet indices

INTRODUCTION

Iron Deficiency Anaemia (IDA) and β-thalassaemia minor (BTM) are considered to be important cause of microcytic hypochromic anaemia. BTM is commonly seen in Mediterranean region, Southwest Europe and Middle East but due to migration and intermixing of different ethnic population this pattern has changed [1]. Although IDA is the most important cause of microcytic hypochromic anaemia in this North Himalayan region of India but cases of BTM are also being reported increasingly and particularly co-existing with IDA [2]. This has led to increase in financial burden involving serum iron studies and haemoglobin electrophoresis along with genetic counseling [3]. Studies have evaluated various red cell parameters which are easily available on electronic cell counters for discrimination of IDA and BTM in different ethnic populations.

The analysis of previously established red cell discriminative indices with new cut off have [4-6] also been done by studies which may be relevant in their set of population for differentiation [3,7]. This variation of cut-offs of various discriminative indices in different populations may be related to mutations in different regions [3].

The present study was conducted to study the previously established red cell discriminative indices in our population and to propose a new modified score in reference to established indices with a new cut-off for early differentiation of IDA and BTM and especially in area where IDA predominates and may co-exist with BTM. In addition, previously all the studies have analysed only the red blood cell parameters but none of them have considered platelet parameters for evaluation. Therefore, it was also intended to formulate a new index taking into consideration all the haematological parameters including RBC and platelet parameters which are available on cell counter that may be helpful as discriminating guide for IDA and BTM.

MATERIALS AND METHODS

This prospective study was conducted over a period of one year from July 2014 till June 2015 which included all the cases of microcytic hypochromic anaemia with MCV< 80 fl presenting in the haematology laboratory of the Pathology Department of Himalayan Institute of Medical Sciences, Dehradun, India after written informed consent was taken from the cases. The male female ratio was 0.2: 1 with age ranging from 2 years to 68 years (median: 28.7 years). These patients were then subjected to serum ferritin and haemoglobin electrophoresis to categorize them into Group I consisting of patients of IDA, group II of BTM, and group III of IDA with BTM (IDA + BTM). Group IV which included patients of anaemia of chronic disorder showed normal or increased serum ferritin with normal haemoglobin electrophoresis and were excluded from the study. The flow chart of the study is shown in [Table/Fig-1]. Serum ferritin was determined on automated analyser (Vidas Bio-merieux, France) and value of HbA2 was determined by high performance liquid chromatography (HPLC) (D-10 Bio-Rad, USA). Cases were diagnosed of IDA when serum ferritin was less than 10 µg/L. BTM was diagnosed when HbA2 was more than 3.5%. The study also included 100 healthy controls which showed MCV within normal range of 80-100 fl.

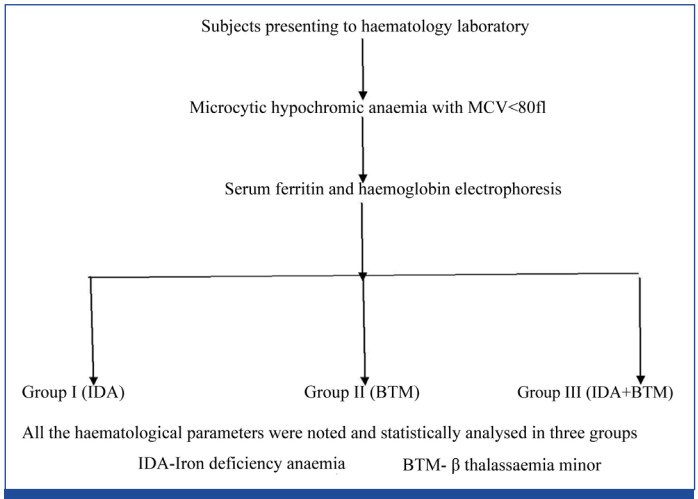
The peripheral blood samples of all the subjects were analysed using fully automated cell counter LH-750 Beckman Coulter (Coulter Corporation, USA). Haematological parameters including Haemoglobin (Hb), Red Blood Cell Count (RBC), Haematocrit (Hct), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Total Leukocyte Count (TLC), Platelet Count (PC), Platelet crit (Pct), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) were noted. Eleven discriminative indices using red blood cell parameters were calculated for group I and group II cases as shown in [Table/Fig-2]. All the cases were kept under category of IDA or BTM according to the established [7-17].

Cut-off for each index and 11T score as published in literature followed by statistical analysis. A new cut off for differentiation of IDA and BTM was proposed in the

study by using ROC curve and analysing AUC which statistically corresponded to highest accuracy. Score 1 was given for every case of BTM and score 0 for IDA and summation of all the scores gave a new modified 11T score. An attempt to formulate a new index using the RBC and platelet parameters was also made for initial discrimination of IDA and BTM. ROC curve analysis with AUC was used for establishing this index.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 20 for windows (Chicago, IL, USA). Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), Youden's Index (YI) and percentage of correctly identified patients were calculated for every index. Area Under Curve (AUC) for each index and the proposed new score were calculated by constructing Receiver Operative Characteristic (ROC) curve. Student t-test was used to compare the various haematological parameters between cases in different groups and controls. The $p<0.05$ was considered statistically significant. The study was approved by the ethical and research committee of the university.



[Table/Fig-1]: Flow chart of the study.

RESULTS

The study included a total of 153 cases with male female ratio of 0.8:1 and mean age of 31.8 years (range 1 to 72 years). Group 1 included 64 cases (IDA), group II 42 cases (BTM), group III 18 cases (IDA+BTM) and remaining 29 cases with neither BTM nor IDA (Anaemia of chronic disorder) were excluded from study. On comparison of mean values of the haematological parameters between BTM cases and controls, it was observed that RBC, Hb, Hct, MCV, MCH, MCHC and PDW showed statistical significant difference ($p<0.05$). [Table/Fig-3] shows the comparison of haematological parameters between group I (IDA) and group II (BTM). It was observed that in addition to RBC parameters, platelet indices also showed statistical significant difference between IDA and BTM. Mean serum ferritin level observed in IDA was 6.87±6.13 µg/L while in BTM was 302.03±366.27 µg/L. Following statistical analysis the new discriminating index was proposed in the present study with formula of RBC x MCHC x MPV/ RDW x PC with cut off of 0.22 to differentiate between IDA and BTM. [Table/Fig-4] shows the evaluation of different indices, 11 T score by Janel et al., along the new modified 11T score and the new index as proposed in the present study [7]. This table shows that amongst all indices new modified 11 T score observed highest sensitivity (87.69%) for IDA while the new index showed highest sensitivity for BTM (77.14%). The correctly identified cases by modified 11T score was 76.85% while that by new index was 75%. [Table/Fig-5] shows the ROC curve with AUC for different indices.

RBC index	Formula	BTM cut off classical published value	Janel et al (2011)	Present study
Mentzer index (MI) [6]	MCV/RBC	<13	<13.3	<14.4
Red cell distribution Width (RDW) [9]	RDW	<14	<17	<19.3
Shine and Lal (SL) [10]	MCVxMCVxMGH/100	<1530	<904	<831.6
England and Fraser (EF) [11]	MCV-(5xHb)-RBC-3.4	<0	<3.6	<10.23
Srivastava (Sr) [12]	MCH/RBC	<3.8	<4	<4.3
Green and King (GK) [13]	MCVxMCVRDW/ Hbz100	<72	<62	<85.7
RDWI	MCVxRDW/RBC	<220	<201	<250.8
Ricerca (Ri) [14]	RDW/RBC	<4.4	<3.1	<4.2
Ehsani (Eh) [15]	MCV-(10xRBC)	<15	<17.3	<19.2
Sirdah (Si) [16]	MCV-RBC-(3xHb)	<27	<30	<32.8
Red blood cell count (RBC) [17]	RBC	>5	>5	>4.43
Janel Score 11T [7]	-	-	>8	-
Modified new 11T	-	-	-	-
Score of present study	-	-	-	>9
New proposed index	RBCxMCHCxMPV	-	-	>0.22
of present study	RDWxPC	-	-	-

[Table/Fig-2]: RBC indices with different cut-off values favouring BTM [7-17].

Haematological parameter	IDA (group I)	BTM (group II)	p-value
	Mean ±SD	Mean ±SD	
RBC ($x10^{12}$/L)	4.12±0.86	4.70±0.97	<0.001
Hb (gm%)	7.97±2.16	9.38±1.92	<0.001
Hct (%)	26.63±6.27	30.12±5.95	<0.001
MCH (pg)	19.30±2.64	20.06±1.96	0.04
MCHC (gm%)	29.70±1.99	31.13±1.11	<0.001
RDW (%)	22.10±4.46	18.57±4.00	<0.001
PC ($x10^9$/L	307.34±160.62	227.29±93.60	<0.001
Pct (%)	0.28±0.11	0.23±0.09	0.01
MPV (fl)	9.46±1.81	10.45±1.93	<0.01
PDW	17.08±0.91	16.67±1.12	0.04

[Table/Fig-3]: Comparison of haematological parameters between group I (IDA) and group II (BTM).
IDA, iron deficiency anaemia; β thalassaemia minor; RBC, Red blood cell count; Hb, haemoglobin; Hct, haematocrit; MCH, mean corpuscular haemoglobin; MCHC, Mean corpuscular haemoglobin concentration; RDW, red cell distribution width; PC, platelet count; Pct, plateletcrit; MPV, Mean platelet volume; PDW, platelet distribution width.

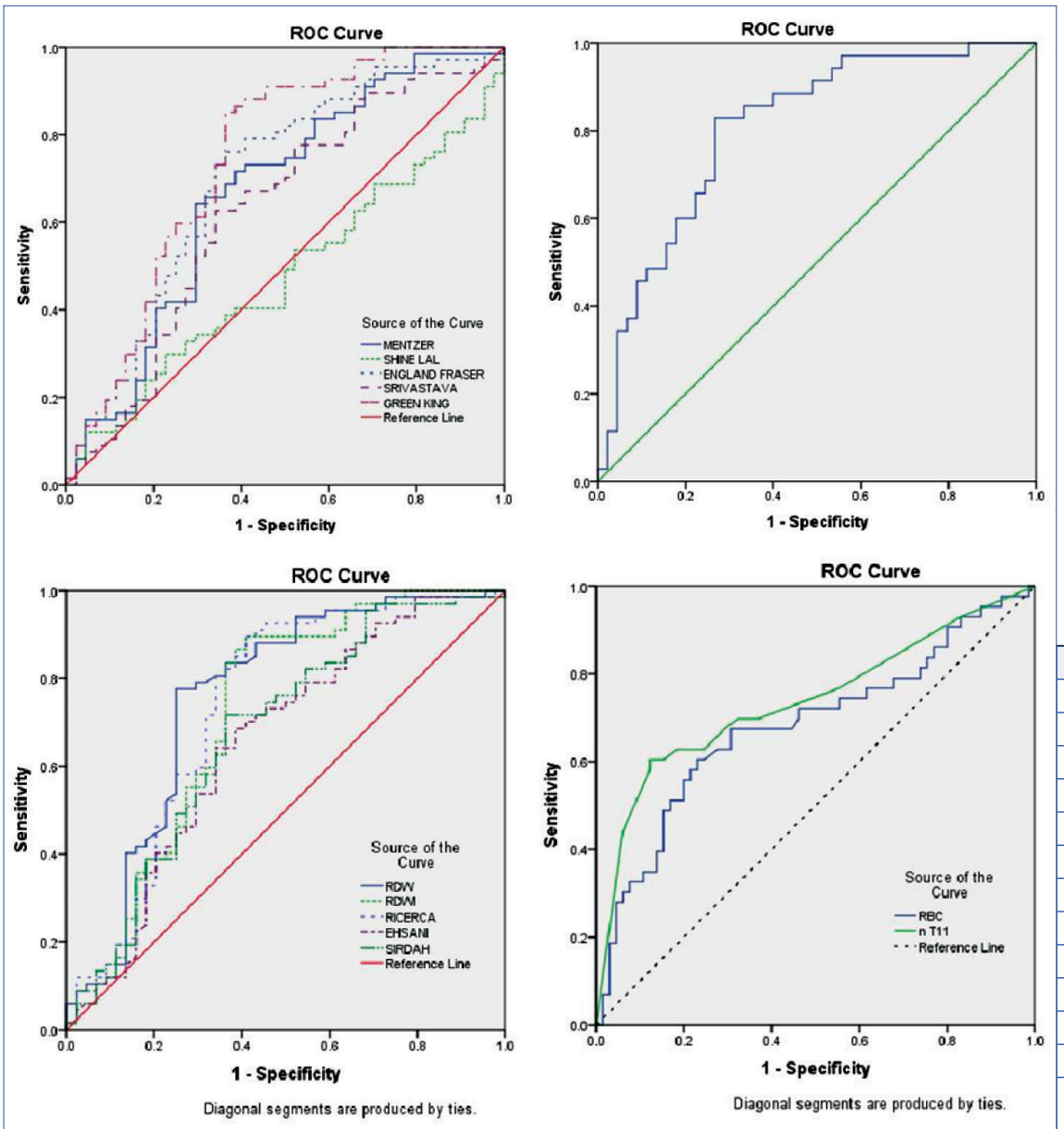
Discriminating index	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden index	Correctly identified cases (%)
MI	69.77	67.19	57.69	76.79	35.92	67.59
RDW	76.74	76.92	68.75	83.33	53.66	76.85
SL	51.16	60	45.83	65.00	11.16	56.48
EF	65.12	78.13	65.12	76.92	42.03	72.22
Sr	58.14	67.19	53.19	70.49	24.29	62.96
GK	62.79	86.15	75.00	77.78	48.94	76.85
RDWI	62.79	86.15	75.00	77.78	48.94	76.85
Ri	67.44	81.25	69.05	78.79	47.44	75.00
Eh	62.79	68.75	56.25	73.33	30.48	65.74
Si	65.12	73.44	60.87	75.81	37.42	69.44
RBC	68.29	73.85	62.22	76.19	38.96	70.37
Janel score 11T	68.29	73.85	62.22	76.19	38.96	70.
Modified new 11T score	65.00	87.69	76.47	77.03	48.15	76.85
New proposed index	77.14	73.33	69.23	80.48	50.47	75

[Table/Fig-4]: Evaluation of established discrimination indices and score with new cut off along with proposed index and score in cases of BTM.
MI, Mentzer index; RDW, Red cell distribution width; SL, Shine and Lal; EF, England and Fraser; Sr, Srivastava; GK, Green and King; RDW 1, Red cell distribution width 1; Ri, Ricerca; Eh, Ehsani; Si, Sirdah; RBC, Red blood cell count

DISCUSSION

BTM and IDA are the important causes of microcytic hypochromic anaemia and their differentiation is important because they not only require different management and treatment but have also different implication to the community or society. BTA requires pre-marriage counselling while IDA needs assessment of nutritional status. 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 [5,6].

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 [3-5] Vehapoglu et al., have concluded in their study from Turkey that Mentzer index has the highest reliability for differentiating BTM and IDA 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 [4,5]. In contrast, in Brazilian and Palestinian population GK index RDWI and Sirdah (Si) Index have proved to be effective for differentiation. The Differences in the effectiveness of various discriminating indices in different [6,16] 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 [3,18].



Index	AUC
Mentzer index	.668
Shine Lal index	.476
England Fraser	.700
Srivastava	.626
Green King	.745
RDW	.756
RDWI	.720
Ricerca	.737
Ehsani	.655
Sirdah	.675
RBC	.682
New modified 11 T	.738
Study Proposed index	.808

[Table/Fig-5]: [Figure/Fig-5]: Receiver operative characteristic curves of different indices along with Area Under Curve (AUC)

In this north Himalayan region of India although IDA is the most important cause of microcytic hypochromic anaemia but due to migration and intermixing of different ethnic populations, BTM is also being reported increasingly and coexisting with IDA [2]. The present study observed that RBC count showed statistical significant difference between groups I (IDA) and III (IDA+BTM) while MCH and MCHC was significantly different in group II (BTM) and III (IDA+BTM). An attempt was made to combine all the previous indices with new cut-off to get modified 11T score which may be useful in differentiating IDA from BTM in this region. The new modified 11T score with cut off of 9 was 87.69% sensitive and specific for IDA and BTM respectively and was able to correctly indentify 76.85% of cases. Janel et al., have also similarly evaluated the diagnostic reliability of 11 red blood cell indices and have concluded that 11T score in their study was able to discriminate between IDA and BTM with high specific and sensitivity and correctly identified 93.02% of patients [7]. However, the present study observed lesser sensitivity and specificity for differentiation of BTM and IDA according to the 11T score as proposed by Janel et al*.,* Therefore it is essential to determine cut-off of the indices by every laboratory for differentiation of IDA and BTM in a given population [7]. An interesting point that has to be highlighted in the study is that apart from red cell parameter, platelet parameters including PC, Pct, MPV and PDW also showed statistical significant difference between IDA and BTM. This has led to the proposal of new formula including the red blood cell and platelet parameters to discriminate between BTM and IDA. The higher MPV observed in BTM may be related to the fact that hyperactive bone marrow in thalassaemia may lead to release of immature platelets in circulation leading to higher MPV.

An important limitation of the present study was that no mutational analysis was carried out in the study. Significant correlation has been reported between β globin gene mutation and MCV as each mutation affects a certain amount of globin chain synthesis resulting in changes in red cell indices [3,19]. Therefore it is essential to further study the various mutations in thalassaemia which are prevalent in this Himalayan region of India which may affect the various RBC parameters and may also have effect on platelet parameters.

CONCLUSION

The proposed new index and modified 11T score may be used for initial discrimination of BTM and IDA especially in this resource limited north Himalayan region of India. Apart from RBC parameters, MPV, PDW and Pct may also be useful in differentiating BTM and IDA. It is essential to determine cut-off of every index in given population for differentiation of these two conditions. Further studies regarding mutational analysis of thalassaemic patients must be carried out in this region.

ACKNOWLEDGEMENTS

This work was supported by grant from Himalayan Institute of Medical Sciences, Swami Rama Himalayan University, Dehradun, India vide letter number HIHTU/HIMS/RC/2013/170.

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FINANCIAL OR OTHER COMPETING INTERESTS: As declared above.

Date of Submission: **Nov 03, 2015**
Date of Peer Review: **Jan 05, 2016**
Date of Acceptance: **Feb 19, 2016**
Date of Publishing: **May 01, 2016**