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Original Research Article

Study of blood indices and high performance liquid chromatography (HPLC) in differentiation of β-thalassemia trait and iron deficiency anaemia

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ABSTRACT

Background: Iron deficiency anemia (IDA) and beta thalassaemia trait (BTT) are two of the most common causes of microcytic anemia.It is essential to differentiate between the two, so as to avoid unnecessary iron therapy which is contraindicated in beta thalassaemia .We retrospectively evaluated the reliability of various indices for differential diagnosis of microcytosis and β-TT in the same patient groups.
Methods: A total of 200 patients were evaluated. We calculated 6 discrimination indices in all patients with anemia or suspected beta thalassemia. None of the subjects had combined IDA and β-TT. The patient groups were evaluated according to the Mentzer, Shine and Lal, England and Fraser, Srivastava, Green and King, Ricerca.
Results: According to percentage correctly diagnosed criteria, Ricerca and Mentzer index are considered as the two best discrimination indices in differentiation between β thalassemia trait and iron deficiency anaemia. Youden's index showed the following ranking with respect to the indices' ability to distinguish between β-TT and IDA, Ricerca index >Mentzer index >Green and King index >Srivastava index >Shine and Lal index >England and Fraser index.
Conclusions: Ricerca and Mentzer index are considered as the two best discrimination indices in differentiation between β thalassemia trait and iron deficiency anaemia.

Keywords: Beta thalassaemia trait, Iron deficiency anemia, Mentzer index, Ricerca index

INTRODUCTION

Iron deficiency is the commonest condition prevalent in most parts of the world. The highest incidence of iron deficiency anaemia is in women during the reproductive period of life. Inadequate iron intake due to poor diet, anorexia (e.g. during pregnancy), diminished bio-availability or impaired absorption may act as contributing factor.

In adult males, the vast majority of cases of iron deficiency are due to chronic haemorrhage, either present or past. In post menopausal women, iron deficiency anaemia is almost invariably due to chronic blood loss, either pathological uterine bleeding, bleeding from the alimentary tract or from other sites. In infants and children, the major etiological factor in causation of iron deficiency anaemia is an inadequate intake of iron in the diet, which fails to meet the increased demands of growth.[1]

Automated cation exchange high performance liquid chromatography (HPLC) has emerged as an excellent screening tool for diagnosing these thalassaemic states.[2]

Haemoglobin fraction analysis by cation-exchange hplc has the advantage of quantifying HbF and HbA_2 along with haemoglobin variant screening in a single, highly reproducible system, making it an excellent technology to screen for haemoglobin variants and haemoglobinopathies along with the thalassaemias.

The simplicity of the automated system makes this an ideal methodology for the routine clinical laboratory. Exact diagnosis of these diseases is of paramount importance in therapy and prevention of genetic transmission.

Iron deficiency anemia and beta thalassaemia trait are two of the most common causes of microcytic anemia.[3] It is essential to differentiate between the two, so as to avoid unnecessary iron therapy which is contraindicated in beta thalassaemia. Beta thalassaemia minor is the most common form of thalassemia.[4] Most homozygotes suffer from a severe syndrome and require regular blood transfusions to survive.

Routine red blood cell counts and other CBC based indices derived from modern blood cell analyzers can be rapidly obtained and are inexpensive.

Various CBC based indices like mentzer index, green and king index, shine and lal index, srivastava index, england and frazer index, ricerca index etc., have been evaluated for screening of BTT.[3-5] These indices can be used as preliminary screening tools which can help distinguish between Iron deficiency anemia(IDA) and Beta thalassemia trait (BTT).[3]

In a tertiary level health care center, it is important to identify suspect BTT individuals from the routine cbc workload. As this involves additional high performance liquid chromatography testing for confirmation of BTT, it is important that a screening index is not only sensitive but also very specific to make it cost effective.

This study was carried out to compare the ability of different indices to distinguish β-TT from IDA and to avoid unnecessary iron therapy which is contraindicated in beta thalassaemia.

METHODS

A total of 200 clinically and haematologically suspected cases of haemolytic anaemia during the period of October 2013 to October 2015 were selected.

Two ml of intravenous blood sample was collected from all cases after obtaining informed consent using Ethylene Diamine Tetra acetic Acid (EDTA) as anticoagulant.

All patients with normal Hemoglobin, but having thalassemic blood indices on coulter (i.e MCV<80, MCH<27), all patients of anemia (OPD and Indoor) showing sickling test positive, patients presenting with hepatosplenomegaly. patients with clinical suspicion of hemolytic anemia, family members of these patients were used as inclusion criteria.

Patient received blood transfusions in last three months were excluded from study.

PS, CBC including RBC Indices, Reticulocyte count, sickling test and if required, bone marrow was done.

Table 1: Analysate identification windows.

Retention time (minutes)	Band (minutes)	Window (minutes)	Range
F	1.15	0.15	1.00-1.30
P2	1.45	0.15	1.30-1.60
P3	1.75	0.15	1.60-1.90
A0	2.60	0.40	2.20-3.30
A2	3.83	0.15	3.68-3.98
D-window	4.05	0.15	3.98-4.12
S-window	4.27	0.15	4.12-4.42
C-window	5.03	0.15	4.88-5.18

Samples were run on HPLC machine Bio-Rad variant-II and hemoglobin graph was obtained and diagnosis of thalassemia was confirmed using values of different hemoglobin fractions and retention times (Table 1).[6]

Special investigations such as iron studies including serum iron, serum ferritin and total iron binding capacity were performed on the sample obtained from the patients to confirm cases of iron deficiency anemia.

RESULTS

In the present study, total 200 suspected cases of Iron deficiency anaemia and Hemoglobin disorders were studied by various CBC based indices and hplc in department of Pathology in Tertiary Care Hospital from October 2013 to October 2015.

We have calculated the number/percentage of correctly diagnosed/ identified patients. Also, we have evaluated the Sensitivity, Specificity, Positive predictive value, Negative predictive value and Youden's Index for different CBC based indices in differentiating β Thalassemia Trait cases from Iron deficiency anaemia. HPLC was considered as standard method.

Out of 200 cases studied, 50 cases (25 %) were diagnosed as having Iron deficiency anemia.

The diagnosis was based on the following features:

- Relevant clinical history: (Stunted growth, hemolytic facies, frontal bossing, pallor, jaundice, pain: small joints / large joints / ribs/ sternum / hip/shoulder / leg ulcers, pain in abdomen rt. upper / lt. upper / epigastric, surgery, if any).

- Low MCV, MCH, MCHC and high RDW (i.e. MCV <80 fl, MCH <27 pg, MCHC <32 g/dl and RDW >46 fl).
- Anisocytosis (predominantly microcytosis), moderate to severe poikilocytosis (ovalocytes, pencil cells, tear drop cells), moderate to severe hypochromia, polychromasia, total and differential leucocyte count and platelet count within normal limit on peripheral blood smear examination.
- Serum iron below normal (i.e.<60 µgm/dl).
- TIBC was increased above normal (i.e.>400 µgm/dl).
- Transferrin saturation decreased (i.e.<15%).

Table 2: Observed hematological parameters and test values in 50 diagnosed iron deficiency anaemia cases.

	Normal range	Observed value	
		Increased value (no. of cases)	Decreased value (no. of cases)
MCV	80-94 fl	0	50
MCH	27-31 pg	0	50
MCHC	32-36 gm/dl	0	50
RDW	29-46 fl	32	18
SI	60-150 µgm/dl	0	50
TIBC	250-400 µgm/dl	50	0
% saturation	20-55 %	0	50

MCV, MCH, MCHC are decreased in all 50 cases diagnosed as Iron deficiency anaemia out of all 200 screened cases. RDW is increased in 32 cases out of 50 cases diagnosed as Iron deficiency anaemia

Serum iron and transferrin % saturation was decreased and total iron binding capacity (TIBC) was increased in all 50 cases diagnosed as Iron deficiency anaemia out of all 200 screened cases.

Table 3: Diagnosis of cases by HPLC in present study.

Diagnosis	Cases
Sickle cell trait (SCT)	43
Sickle cell disease (SCD)	9
Sickle cell - beta thalassemia (SBT)	18
Beta thalassemia trait (BTT)	32
Beta thalassemia major (BTM)	16
Hbe - beta thalassemia	1
Hb lepore trait	1
Normal pattern	80

In the present study HPLC was considered as standard method.

So total studied cases include:

- Hemoglobinopathies - 120 cases
- Iron deficiency anaemia - 50 cases
- Anaemia due to systemic diseases - 30 cases

A total of 30 cases suspected to be Iron deficiency anaemia / Hemoglobinopathies were found to be normal by hplc.

They showed the serum iron, TIBC and transferrin saturation percentage within normal range. These 30 cases were taken as normal control group.

Table 4: Groupwise classification of hemoglobin disorders.

Group	Diagnosis	No. of cases
A	Sickle cell trait (SCT)	43 (35.83%)
B	Sickle cell disease (SCD)	9 (7.5%)
C	Sickle cell - beta thalassemia (SBT)	18 (15%)
D	Beta thalassemia trait (BTT)	32 (26.66%)
E	Beta thalassemia major (BTM)	16 (13.33%)
F	Hemoglobin E-Beta thalassemia	1 (0.83%)
G	Hb lepore trait	1 (0.83%)
	Total cases of hemoglobin disorders	120

120 cases were diagnosed as Hemoglobin disorders out of 200 cases by hplc, so cases were classified as:

- Group-A Sickle Cell Trait (SCT)
- Group-B Sickle Cell Disease (SCD)
- Group-C Sickle Cell - Beta Thalassemia (SBT)
- Group-D Beta Thalassemia Trait (BTT)
- Group-E Beta Thalassemia Major (BTM)
- Group-F HbE - Beta Thalassemia
- Group-G Hb Lepore Trait
- Group-H Iron deficiency anaemia

Table 5: Mean hematological parameters in β-TT and IDA.

Parameters	BTT (Mean±SD)	IDA (Mean±SD)
HB	10.21±1.79	7.88±2.54
RBC	5.06±0.38	4.07±1.11
MCV	66.84±6.34	70.31±7.25
MCH	20.02±2.31	19.21±3.22
MCHC	29.90±1.83	27.17±2.60
RDW	15.73±1.36	44.31±10.62

Hb values in the β-TT group were 10.21±1.79 and those in the IDA group were 7.88±2.54. MCV was 66.84 ±6.34 in the β-TT group and these values were lower in the IDA group i.e.70.31±7.25.

RBC count was higher in the β-TT (5.06±0.38) group than that in the IDA group (4.07±1.11). MCH was higher in the β-TT (20.02±2.31) group than that in the IDA group (19.21±3.22).

MCHC was higher in the β-TT (29.90±1.83) group than that in the IDA group (27.17±2.60). RDW was lower in

the β-TT (15.73±1.36) group than that in the IDA group (44.31±10.62).

Table 6: Different RBC indices and mathematical formulas used to differentiate between β-TT and IDA.

Hematological index	Formula
Mentzer Index (MI)	MCV/RBC
Shine and Lal Index (S and L)	MCV×MCV×MCH/100
Srivastava Index(SRI)	MCH/RBC
Green and King Index (G and K)	MCV×MCV×RDW/Hb×100
England and Fraser Index (E and F)	MCV−(5×Hb)−RBC−3.4
Ricerca Index	RDW/RBC

Sensitivity = [True Positive/(True Positive+False Negative)] × 100

Specificity = [True Negative/(True Negative + False Positive)] × 100

Positive Predictive Value = [True positive/ (True Positive + False Positive) × 100

Negative Predictive Value = [True Negative/ (True Negative + False Negative) × 100

Youden's index = [(Sensitivity + Specificity) – 100].

Table 7: Threshold values of the Indices used to discriminate between β-TT and IDA.

Index	Iron deficiency anaemia	Beta thalassemia trait
Mentzer index (MI)	>13	≤13
Shine and Lal index (S and L)	>1530	≤1530
Srivastava index(SRI)	>0	≤0
Green and King index (G and K)	>3.8	≤3.8
England and Fraser index (E and F)	>4.4	≤4.4
Ricerca index (RIC)	>65	≤65

Mentzer Index (MI)

If the quotient is less than 13 or equal to, Thalassemia is said to be more likely. If greater than 13, then iron-deficiency anemia is said to be more likely.

Shine and Lal Index (S and L)

A value greater than 1530 indicated iron deficiency and value less than or equal to 1530 indicated β-thalassemia trait.

Srivastava Indexa (SRI)

A value greater than zero indicated iron deficiency and a value less than or equal to zero indicated β-thalassemia trait.

Green and King Index (G and K)

A value greater than 3.8 indicated iron deficiency and a value less than or equal to 3.8 indicated β-thalassemia trait.

England and Fraser Index (E and F)

A value greater than 4.4 indicated iron deficiency and a value less than or equal to 4.4 indicated β-thalassemia trait.

Ricerca Index (RIC)

A value greater than 65 indicated iron deficiency and a value less than or equal to 65 indicated β-thalassemia trait.

Table 8: Differential values of CBC based indices and correctly identified number of patients.

	BTT (n=32)	IDA (n=50)	Total correctly identified patients(n=82)	% of correctly identified patients
MI				
BTT	23	9	64	78.0
IDA	9	41		
S&L				
BTT	31	50	31	37.8
IDA	1	0		
E and F				
BTT	0	2	48	58.5
IDA	32	48		
SRI				
BTT	12	14	48	58.5
IDA	20	36		
RIC				
BTT	32	2	80	97.5
IDA	0	48		
G and K				
BTT	12	1	61	74.3
IDA	20	49		

The Table 8 reveals that percentage of correctly diagnosed patients is highest for the Ricerca index (97.5 %) which is closely followed by Mentzer Index (78%). The third highest was Green and Kings Index (74.3%).

As indicated in Table 9, Ricerca Index, Shine and Lal index demonstrated the highest sensitivity of 100% and 96.8% respectively and specificities for correctly identifying IDA (100%, 96.8%) and β-TT (96%, 0%).

The Green and King, England and Fraser and the Mentzer indices demonstrated the highest specificities at 98% and 96% and 82% respectively.

Furthermore, Table 9 shows the highest and lowest PPV, which were found for the Ricerca index (94%) and the England and Fraser index (0%), respectively.

Table 9: Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and Youden's index of indices to differentiate between β-TT and IDA.

Indices	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
MI					
BTT	71.8	82	71.8	82	53.8
IDA	82	71.8	82	71.8	
S and L					
BTT	96.8	0	62	0	-3.2
IDA	0	96.8	0	62	
E and F					
BTT	0	96	0	60	-4
IDA	96	0	60	0	
SRI					
BTT	37.5	72	46	64	9.5
IDA	72	37.5	64	46	
RIC					
BTT	100	96	94	100	96
IDA	96	100	100	94	
G and K					
BTT	37.5	98	92	71	35.5
IDA	98	37.5	71	92	

Ricerca index demonstrated the highest NPV at 100% and the Shine and Lal index had the lowest NPV at 0%.

Additionally, above table shows that the highest and lowest Youden's index values belonged to the Ricerca index (96%) and the England and Fraser index (-4%).

Youden's index showed the following ranking with respect to the indices' ability to distinguish between β-TT and IDA: Ricerca index >Mentzer index >Green and King index >Srivastava index >Shine and Lal index >England and Fraser index

DISCUSSION

The present study was carried out in department of pathology in a tertiary care hospital from October 2013 to 0ctober 2015.

A discriminant formula or CBC based index with a combination of high sensitivity and specificity for detecting BTT would be a very useful tool in the investigation of microcytic hypochromic anemia in countries and areas where nutritional deficiencies and thalassemia are present with higher prevalence.

Various CBC based indices like Mentzers Index, Green and King Index, Shine and Lal Index, Srivastava Index, England and Frazer Index, Ricerca Index etc., have been published and evaluated for screening of BTT.[3-5.]

These indices can be used as preliminary screening tools which can help to distinguish between Iron deficiency anaemia and Beta thalassemia trait and to allow confirmation by standard test like high performance liquid chromatography.

In the present study, percentage of correctly identified patients is highest for the Ricerca index (97.5 %) which is closely followed by Mentzer Index (78 %). The third highest was Green and Kings Index (74.3 %).

Ehsani MA et al studied the percentage of correctly identified patients is highest for Mentzer index (94.71 %) followed by their new index (92.96 %).[7]

Demir et al studied the percentage of correctly identified patients is highest for the Shine and Lal index (92%).[8]

Mussarrat N et al they found percentage correctly diagnosed is highest for RDW index (88.14%).[9] Matos JF G and K correctly diagnosed the greatest number of patients (297/336,88.39 %).[10]

Aysel V et al studied the percentage of correctly diagnosed patients was the highest with the Mentzer

index (91%).[11] After comparison with other studies the Ricerca index which shows 94% Positive predictive value in differentiating iron deficiency anaemia and beta thalassemia trait was employed by Tzu- Huang C et al who found to give positive predictive value of 94%, followed by Mussarrat et al (80%), TP, SA et al (77.7%), Okan V et al (75.3%), Keikhaei B et al (72.6%), Vehapoglu A et al (57%).[9,11-15] Rest of authors did not quote their findings of Ricerca index.

Table 10: Differential values of CBC based indices and correctly identified number of patients.

Studies		MI	S and L	E and F	SRI	RIC	G and K
Ehsani MA et al[7]	Correctly identified cases out of total cases	269/284	-	236/284	247/284	-	-
	Percentage	94.71%	- %	83.09%	86.97%	- %	- %
Demir et al[8]	Correctly identified cases out of total cases	152/173	160/173	147/173	153/173		146/173
	Percentage	88 %	92 %	85 %	89 %	%	84 %
Mussarrat N et al[9]	Correctly identified cases out of total cases	271/312	226/312	238/312	257/312	251/312	262/312
	Percentage	86.85%	72.43%	78.28%	82.37%	80.44%	83.97%
Matos JF et al [10]	Correctly identified cases out of total cases	290/336	66/336	-	289/336	-	297/336
	Percentage	86.30%	19.64%	-	86.01%	-	88.39 %
Aysel V et al[11]	Correctly identified cases out of total cases	264/290	168/290	218/290	230/290		228/290
	Percentage	91 %	57.9 %	75 %	79.3 %	%	78.6 %
Present study	Correctly identified cases out of total cases	64/82	31/82	48/82	48/82	80/82	61/82
	Percentage	78.0 %	37.8 %	58.5 %	58.5 %	97.5 %	74.3 %

Table 11: Observed values of sensitivity of different blood Indices by different authors and in present study in differentiation of β-TT and IDA.

Studies	RIC %	MI %	G and K %	SRI %	S and L %	E and F %
Ehsani MA et al[7]	-	94.6	-	85.7	-	69.5
Okan Vet al[12]	98	82	83	74	91	78
Demir et al[8]	-	93	83	81	100	71
Bijan Keikhaei et al[13]	98	79.4	94.3	71.1	96.4	97.4
Niazi M et al[9]	92	89	86	61	72	91
TP,SA et al [14]	71.1	82.9	72.5	92.5	99.2	79.2
Januária Fonseca Matos et al[10]	-	76.6	80.9	55.3	100	-
Aysel Vehapoglu et al[11]	100	98.7	83.1	85.7	100	66.2
Tzu-Chuan Huang et al[15]	77	67	83	57	100	74
Kunal Sehgal et al[16]	75.6	76.9	79.5	88.5	94.9	52.5
Present Study	100	71.8	37.5	37.5	96.8	0

Thus, after comparison with other studies the Ricerca index which shows 100% sensitivity in differentiating iron deficiency anaemia and beta thalassemia trait was employed by Vehapoglu A et al who found to give Sensitivity of 100%, followed by Keikhaei B et al (98%), Okan V et al (98%), Mussarrat et al (92%), Tzu-Chuan H et al (77%), Sehgal K et al (75.6%).[9,11-13,15,16] Rest of authors did not quote their findings of Ricerca index.

After comparison with other studies the Ricerca index which shows 100% negative predictive value in differentiating iron deficiency anaemia and beta

thalassemia trait was employed by Vehapoglu A et al who found to give negative predictive value of 100% followed Okan V et al (97.1%), Keikhaei B et al (92.8%), Mussarrat et al (82%), Tzu-Chuan H et al (73%), TP SA et al (32.2%).[9,11-15] Rest of authors did not quote their findings of Ricerca index.

Table 12: Observed values of specificity of different blood indices by different authors and in present study in differentiation of β-TT and IDA.

Studies	RIC %	MI %	G and K %	SRI %	S and L %	E and F %
Ehsani MA et al[7]	-	95.5	-	88.5	-	69.5
Okan V et al[12]	14	78	96	79	100	97
Demir et al[8]	-	85	85	92	89	92
Keikhaei B et al[13]	41	95.8	90.9	92.7	35	85.1
Niazi M et al[9]	62	81	78	66	100	56
TP, SA et al[14]	76.5	80.2	70.3	46.9	29.6	90
Matos JF et al[10]	-	87.9	89.6	91	6.6	-
Vehapoglu A et al[11]	14.7	82.3	73.5	72	10.2	85.3
Tzu-Chuan H et al[15]	92	90	94	84	91	94
Sehgal K et al[16]	44.4	87	66.7	80.4	56.5	86.9
Present Study	96	82	98	72	0	96

Table 13: Observed values of positive predictive value of different blood indices by different authors and in present study in differentiation of β-TT and IDA.

Studies	RIC %	MI %	G and K %	SRI %	S and L %	E and F %
Okan V et al[12]	75.3	71.9	100	56.9	98.6	100
Demir et al[8]	-	76	74	84	82	82
Keikhaei B et al[13]	72.6	96.8	96.2	93.9	70.3	98.1
Niazi M et al[9]	80	93	93	83	100	74
TP,SA et al[14]	77.7	85.4	86.9	87.9	88.8	91.3
Matos JF et al[10]	-	7.7	9.3	7.5	1.4	-
Vehapoglu A et al[11]	57	86.3	77.6	77.6	55	83.6
Tzu-Chuan H et al[15]	94	94	95	95	90	96
Present study	94	71.8	92	46	62	0

Youden's index showed the following ranking with respect to the indices' ability to distinguish between β-TT and IDA: Ricerca index >Mentzer index >Green and King index >Srivastava index >Shine and Lal index >England and Fraser index.

Thus, after comparison with other studies the Ricerca index which shows 96% Youden's index value in differentiating iron deficiency anaemia and Beta thalassemia trait was employed Tzu-Chuan H et al who found to give Youden's index value 70% followed by Mussarrat et al (50%), TP, SA et al (47.6%), Keikhaei B et al (39%), Sehgal K et al (20%), Vehapoglu A et al (14.7%), Okan V et al (12%).[9,12-16] Rest of authors did not quote their findings of Ricerca index.

In study of Ehsani MA et al, the percentage of correctly identified patients is highest for Mentzer index (94.71%).[7]

Okan V et al studied the S and L and G and K indices were the best indicators in both comparisons and the RDW index was the worst.[12]

Demir et al found the most accurate discriminative index for patients younger than 10 years was Shine and Lal and for those older than 10 years it was RDW index.[8]

Keikhaei B et al, According to Youden's index (YI), Discriminating Functions in the order of highest to lowest were KI >G and KI > RDWI >E and FI >RBC>M I>EI >TI >SI >S and BI >RI>S and LI >B and FI.[12]

Mussarrat et al, found RDWI is leading.[9]

TP, SA et al Youden's index in decreasing order was as follows: RDW-SD > RBC COUNT> E and F >MI >RI >RDWI >G and K >SI >S and L >RDWCV.[14]

Januária Fonseca Matos et al studied the most reliable indices were GKI and RDWI, while SLI presented the lowest accuracy.[10] Vehapoglu A et alYouden's index showed the following ranking: Mentzer index >Ehsani et al. index >RBC count > Sirdah et al. index >RDWI >Srivastava index >Green and King index >England and Fraser index >MDHL >Ricerca et al. index >Shine and Lal index >MCHD.[11]

Table 14: Observed values of negative predictive value of different blood indices by different authors and in present study in differentiation of β-TT and IDA.

Studies	RIC %	MI %	G and K %	SRI %	S and L %	E and F %
Okan V et al[12]	97.1	79	85.4	62.8	91.5	81.9
Demir et al[8]	-	96	90	91	100	86
Keikhaei B et al[13]	92.8	74.5	86.6	66.8	86	80.4
Niazi M et al[9]	82	71	78	81	3	82
TP,SA et al[14]	32.2	46.4	46.6	72.2	48.8	33.9
Matos JF et al[10]	-	99.6	99.7	99.4	100	-
Vehapoglu A et al[11]	100	98.2	79.3	81.6	100	69
Tzu-Chuan H et al[15]	73	54	80	29	100	66
Present study	100	82	71	64	0	60

Table 15: Observed values of Youden's Index of different blood indices by different authors and in present study in differentiation of β-TT and IDA.

Studies	RIC %	MI %	G and K %	SRI %	S and L %	E and F %
Ehsani MA et al[7]	-	90.1	-	74.2	-	68.7
Okan V et al[12]	12	60	79	53	91	75
Demir et al[8]	-	79	68	73	89	63
Keikhaei B et al[13]	39	75.3	85.2	63.8	31.4	82.6
Niazi M et al[9]	50	70	64	27	72	47
TP, SA et al[14]	47.6	63.2	42.8	39.4	28.8	69.3
Matos JF et al[10]	-	64.5	70.4	46.3	6.6	-
Vehapoglu A et al[11]	14.7	81	56.6	57.7	10.2	51.4
Tzu-Chuan H et al[15]	70	58	77	41	91	68
Sehgal K et al[16]	20	64	46.2	68.9	51.4	39.5
Present Study	96	53.8	35.5	9.5	-3.2	-4

Sehgal K et al found that the new index "Sehgal index" and MI <14 met the requirements of both high sensitivity and high specificity to predict BTT irrespective of iron levels in a large number of patients studied both retrospectively and prospectively.[16]

CONCLUSION

According to percentage correctly diagnosed criteria Ricerca and Mentzer index are considered as the two best discrimination indices in differentiation between β thalassemia trait and Iron deficiency anaemia.

Youden's index showed the following ranking with respect to the indices' ability to distinguish between β-TT and IDA: Ricerca index >Mentzer index >Green and King index >Srivastava index >Shine and Lal index >England and Fraser index.

These functions that are calculated when routine blood counts are done by blood analyzers, are less time-consuming methods and less expensive than the standard tests for diagnosing these disorders.

Cell counter–based formulas particularly Ricerca, Mentzer and Green and King index have good discriminative function compared with other.

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REFERENCES

1. Wintrobe MM, Lee GR. Wintrobe's Clinical Hematology. 10th ed. 1998.
2. Rangan A, Handoo A, Sinha S, Saxena R, Verma IC, Kumar S et al. Utility of family studies in

diagnosing abnormal hemoglobins/ thalassemic states. Indian J Pediatr. 2009 June;76(6):615-21.
3. Akai Y, Kubota F, Bichile SK, Mehta NB. A Study of beta thalassemia screening using an automated hematology analyser. Symex J int. 1998;8(2).
4. Cartwright GE, Lee GR. The anaemia of chronic disorders. Br J Hematol. Br J Haematol. 1971 Aug;21(2):147-52.
5. Bainton DF, Finch CA. The diagnosis of iron deficiency anemia. Am J Med. 1964 Jul 1;37(1):62-70.
6. Biorad: Instruction manual 2006. Variant Beta-thalassemia short program. Available at: http://www.bio-rad.com/ en-us/ product/ hemoglobinopathies/ variant-ii-beta-thalassemia-short-program-reorder-pack.
7. Ehsani MA, Shahgholi E, Rahiminejad MS, Seighali F, Rashidi A. A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients. Pak J Biol Sci: PJBS. 2009 Mar;12(5):473-5.
8. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. Turk J Hematol. 2009 Sep 1;26(3):138-45.
9. Niazi M, Tahir M, e Raziq F, Hameed A. Usefulness of Redcell Indices in Differentiating Microcytic Hypochromic Anemias. Gomal J Med Sci. 2010 Dec 31;8(2).
10. Matos JF, Dusse LM, Stubbert RV, Ferreira MR, Coura-Vital W, Fernandes AP et al. Comparison of discriminative indices for iron deficiency anemia and β thalassemia trait in a Brazilian population. Hematology. 2013 May 1;18(3):169-74.
11. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency anemia. Anemia. 2014 Apr 10;2014.
12. Okan V, Cigiloglu A, Cifci S, Yilmaz M, Pehlivan M. Red cell indices and functions differentiating patients with the β-thalassaemia trait from those with iron deficiency anaemia. J Int Med Res. 2009 Feb;37(1):25-30.
13. Keikhaei B. A new valid formula in differentiating iron deficiency anemia from ß-thalassemia trait. Pak J Med Sci. 2010;26:368-73.
14. TP, SA. Discriminant functions in distinguishing beta thalassemia trait and iron deficiency anemia: the value of the RDW-SD. Internet J Hematol. 2010;7(2).
15. Huang TC, Wu YY, Chen YG, Lai SW, Wu SC, Ye RH et al. Discrimination index of microcytic anemia in young soldiers: a single institutional analysis. PloS one. 2015 Feb 13;10(2):e0114061.
16. Sehgal K, Mansukhani P, Dadu T, Irani M, Khodaiji S. Sehgal index: A new index and its comparison with other complete blood count-based indices for screening of beta thalassemia trait in a tertiary care hospital. Indian J Pathol Microbiol. 2015;58(3):310.

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