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Discriminant Indices for distinguishing Beta Thalassemia Trait (BTT) and Iron Deficiency Anemia (IDA): Workup at microcytic hypochromic anemia

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Abstract

Context: The microcytic erythrocytosis is largely confronted by the clinicians for its underlying etiology, either as iron deficiency anemia or beta thalassemia trait. The morphological differentiation between IDA and BTT is difficult on the peripheral smear examination and by cell counter studies. Therefore, a study was planned for MCHC anemia to be assessed by 6 commonly used indices worldwide for their discrimination capacities of IDA versus BTT.

Aims: The aim was to study six popular discriminant red blood cell indices to distinguish the iron deficiency anemia from beta thalassemia trait presenting as microcytic hypochromic anemia.

Settings and Design: Observational and analytical.

Methods and Material: Present study included 50 patients each in group A (IDA) and group B (BTT) based on the results of CBC, peripheral smear examination, S. Ferritin and HbA_2 analysis. Six indices were evaluated in the present study, Bessman index [RDW], Mentzer index, Srivastava index, Green and King index, Sirdah index and Ehsani index.

Statistical analysis used: The statistical tests for sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios, accuracy and Youden's index were used to compare the six indices amongst groups A and B.

Results: Group A included 12 males and 38 females (male to female ratio of 1: 3.2) and mean age of 25.02±11.24 years. Group B included 14 males and 36 females (male to female ratio of 1: 2.6) and mean age of 21.38±11.73 years. MI and EI showed highest values for Accuracy and YI when compared for screening of IDA and BTT patients, while BI [RDW] performed poorly for both the groups.

Conclusions: MI and EI can be reliably used for screening of MCHC anemia patients to categorise them to IDA or BTT.

Keywords: Microcytic erythrocytosis, discriminant indices, beta thalassemia trait, iron deficiency anemia

Introduction

Microcytic erythrocytosis is a commonly reported red blood cell pathology in the clinical hematological laboratories in hospitals. Rather microcytic hypochromic (MCHC) anemia, a consequence to a varied underlying etiology, which is frequently encountered by pathologists. Fifty eight percent of children (aged 6-59 months), 53% of women and 23% of men (aged 15-49 years) in Indian population have been reported to suffer from anemia in the survey of National Family Health Survey (NFHS)-4 (2015-16) [1]. The common type of anemia reported in the survey is microcytic, attributed to a state of iron deficiency. MCHC anemia is the consequence of iron deficiency, beta thalassemia trait (BTT) or the combination of these along with few other less frequently encountered causes, that include hemoglobinopathies [2].

The microcytic erythrocytosis, synonymous to MCHC anemia, is largely perceived by the clinicians for its underlying etiology, either a state of iron deficiency or BTT. The morphological differentiation between the iron deficiency anemia (IDA) and BTT is difficult on the peripheral smear examination and also to some extent by automated cell counters [3-6]. The investigative approach to know the underlying etiology of MCHC anemia is to evaluate serum iron studies, the study of Hb electrophoresis or the chromatography (HPLC) [7-10]. However, such studies require the advanced clinical laboratory setups.

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Therefore, to distinguish between IDA and BTT before proceeding to high end investigations, the simple screening methods are necessary. These methods would help in segregating the patients of MCHC anemia further to undergo the specialised investigations for confirmation that would reduce the unnecessary sampling burden on clinical hematology laboratories.

IDA and BTT have been attempted to be separated over past many decades by a number of red blood cell indices. These indices are the mathematical calculations or proportions between the various morphometric and quantitative values of red blood cells [11-15].

There are about 40 such mathematical indices which have been proposed in hematological literature search for IDA and BTT in the subjects of MCHC anemia [16].

There are studies for the utilization of these indices to be used as a screening tool to suggest and segregate the population of MCHC anemia to its underlying etiologies, but not without the contrasting values of sensitivity and specificity for these indices [3, 5, 6, 12, 17-12].

This study was planned in the backdrop of above scenario of MCHC anemia, largely for prevailing anemia to be assessed by 6 commonly used indices worldwide for their discrimination capacities of IDA versus BTT. The aim was to study the discriminant red blood cell indices to distinguish the iron deficiency anemia from beta thalassemia trait presenting as microcytic hypochromic anemia.

Subjects and Methods

Patients

This observational and analytical study was carried out for a period of two years and a total of 100 MCHC anemia patients were included in this study based on the complete blood count (CBC) with MCV <70 fL and peripheral smear examination showing microcytosis and hypochromia. The patients were divided into two groups. Group A included IDA patients (n = 50) and were diagnosed on the basis of Hb <13 g% and S. ferritin <30 ng/ml for adult males, Hb <12 g% and S. ferritin < 13 ng/ml for adult females, and Hb <11 g% and S. ferritin <10 ng/ml for pediatric population. Group B included BTT patients (n = 50) and were diagnosed on the basis of HbA_2 >3.5% by HPLC. Patients with MCV >70 fL, hemoglobinopathies other than BTT, on treatment for iron deficiency, chelating therapy or blood transfusion were excluded from the study.

The values of Hb, RBC count, MCV, MCH, MCHC and RDW were obtained using automated cell counter.

Discriminant Indices

The six discriminant indices evaluated in the study with their formulae for calculation and cut off values for each group are summarised in table 1.

Table 1: Discriminant Indices with their formulae and cut off values (RDW-Red cell Distribution Width, MCV-Mean Corpuscular Volume, RBC-Red Blood Cell, MCH-Mean Corpuscular Hemoglobin, Hb-Hemoglobin)

Discriminant Indices	Formulae	IDA (Group A) Cut Off Value	BTT (Group B) Cut Off Value
1. Bessman (BI) [21]	RDW	>14	< 14
2. Mentzer (MI) [22]	MCV/RBC	>13	< 13
3. Srivastava (SI) [23]	MCH/RBC	>3.8	< 3.8
4. Green & King (G&K) [24]	MCV x MCV x RDW/Hb x 100	>65	< 65
5. Sirdah (Si) [25]	MCV-(5 x Hb)-RBC-3.4	>27	< 27
6. Ehsani (EI) [9]	MCV-(10RBC)	>15	< 15

These indices were evaluated and compared based on their sensitivity (SN), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio, negative likelihood ratio, accuracy and Youden's index (YI) in patients of MCHC anemia.

Results

Group A constituted of 50 IDA patients with 12 males and 38 females (male to female ratio of 1: 3.2) and mean age of 25.02±11.24 years. Group B constituted of 50 BTT patients with 14 males and 36 females (male to female ratio of 1: 2.6) and mean age of 21.38±11.73 years. The mean value of S. ferritin observed in adult males was 22 ng/ml, 11 ng/ml for adult females and 6.2 ng/ml for pediatric population in group A. Group B observed mean HbA_2 of 5.3±0.4%. The mean values with standard deviations for the red cell parameters observed in both the groups are given in table 2.

Table 2: Hematological parameters with their mean values (IDA-Iron Deficiency Anemia, BTT-Beta Thalassemia Trait, MCV-Mean Corpuscular Volume, MCH-Mean Corpuscular Hemoglobin, MCHC-Mean Corpuscular Hemoglobin Concentration, RDW-Red cell Distribution Width)

Parameters	Group A (IDA)	Group B (BTT)
1. MCV (fL)	65.41±4.69	61.92±5.61
2. MCH (pg/cell)	20.53±3.46	18.65±2.75
3. MCHC (g/dl)	30.03±1.96	30.01±2.67
4. RDW	17.524±3.02	17.828±2.88

Number of correctly identified patients for both the groups are summarised in table 3 and based on the statistics observed, the six indices are compared in table 4. According to the results of present study. In group A, highest SN was obtained by Si index (100%), highest SP by EI (48%), highest PPV by EI (64.86%), highest NPV by Si index (100%), highest PLR by EI (1.846) and lowest NLR by MI (0.043). In group B, highest SN was obtained by EI (48%), highest SP by Si index (100%), highest PPV by Si index (100%), highest NPV by EI (64.86%), highest PLR by MI (23) and lowest NLR by EI (0.542). MI (72% and 0.44) and EI (72% and 0.44) showed the highest rate of accuracy and YI, followed by SI (69% and 0.36), and lowest performance was observed with BI [RDW] (49% and-0.02) for differentiation of IDA and BTT from the cases of MCHC anemia. A p-value of 0.0001 was considered statistically significant.

Table 3: Number and percentage of correctly identified patients (IDA-Iron Deficiency Anemia, BTT-Beta Thalassemia Trait)

Serial number	Index	Groups (n = 50)	Number of correctly identified patients	Percentage of correctly identified patients (%)
1	Bessman [RDW]	A (IDA)	45	90
		B (BTT)	4	8
2	Mentzer	A (IDA)	49	98
		B (BTT)	23	46
3	Srivastava	A (IDA)	48	96
		B (BTT)	20	40
4	Green & King	A (IDA)	49	98
		B (BTT)	11	22
5	Sirdah	A (IDA)	50	100
		B (BTT)	16	32
6	Ehsani	A (IDA)	48	96
		B (BTT)	24	48

Table 4: Statistical comparison of six indices (S.D.-Standard Deviation, SN-Sensitivity, SP-Specificity, PPV-Positive Predictive Value, NPV-Negative Predictive Value, PLR-Positive Likelihood Ratio, NLR-Negative Likelihood Ratio, YI-Youden's Index, IDA-Iron Deficiency Anemia, BTT-Beta Thalassemia Trait, S-Significant, NS-Non Significant)

INDEX	GROUP	MEAN ± S.D	SN (%)	SP (%)	PPV (%)	NPV (%)	PLR	NLR	Accuracy (%)	YI	P-VALUE
Bessman [RDW]	A (IDA)	17.52±3.02	90	8	49.45	44.44	0.97	1.25	49	-0.02	0.60, NS
	B (BTT)	17.82±2.88	8.51	90	44.44	51.14	0.851	1.016			
Mentzer	A (IDA)	20.94±8.67	98	46	64.47	95.83	1.814	0.043	72	0.44	0.0001, S
	B (BTT)	13.05±1.85	46	98	95.83	64.47	23	0.55			
Srivastava	A (IDA)	6.21±2.58	96	40	61.54	90.91	1.6	0.1	69	0.36	0.0001, S
	B (BTT)	3.92±0.57	40	96	90.91	61.54	10	0.625			
Green & King	A (IDA)	124.65±58.97	98	22	55.68	91.67	1.256	0.09	60	0.2	0.0001, S
	B (BTT)	77.63±17.49	22	98	91.67	55.68	11	0.796			
Sirdah	A (IDA)	43.02±8.46	100	32	59.52	100	1.47	N.A.	66	0.32	0.0001, S
	B (BTT)	30.12±6.44	32	100	100	59.52	N.A.	0.68			
Ehsani	A (IDA)	34.20±13.95	96	48	64.86	92.31	1.846	0.083	72	0.44	0.0001, S
	B (BTT)	14.92±8.51	48	96	92.31	64.86	12	0.542			

Discussion

The discrimination of IDA and BTT, the most common etiologies of MCHC anemia, have been attempted using red cell parameters since 1970's [21-24]. Since then, numerous other mathematical indices have been introduced, which in original publications prove to work well with near 100% SN, but failed to achieve the same results in subsequent evaluations [5, 11, 12, 26]. The differentiation of MCHC anemia patients is imperative to start appropriate treatment for either etiology [4, 10, 17]. The newer technologies, like iron studies and chromatography, though are confirmatory, but are not readily available in developing country like India, where the thalassemia trait is quite prevalent [8, 26-27].

Therefore, this study was carried out with the aim of evaluating the discriminant red blood cell indices to distinguish the iron deficiency anemia from beta thalassemia trait presenting as microcytic hypochromic anemia. The indices (BI, MI, SI, G&K index, Si index and EI) were assessed in laboratory confirmed cases of IDA (group A, n = 50) and BTT (group B, n = 50) based on low MCV, MCHC picture on peripheral smear, low S. ferritin for IDA patients and HbA_2 >3.5% for BTT patients. Female preponderance was observed in both the groups (1:3.2 in group A and 1:2.6 in group B). Similar distribution of gender was also noted by Ntaios *et al.* [5], Janel *et al.* [7], Okan *et al.* [10] and Zaghloul *et al.* [19] in their studies.

The present study also compared the hematological parameters in both the groups and observed a higher set of mean values for MCV and MCH, however MCHC and RDW did not show much difference among the two groups, as described in table 2. AlFadhli *et al.* [4] and Trivedi *et al.* [15] also observed similar results for red cell parameters. The mean values for each index was also calculated, depicted in table 4, all showed statistical difference except BI[RDW]. Bhargava *et al.* [28] observed mean values of 17.6±3.2 for MI, 6.1±1.3 for SI, 117.2±37.6 for G&K index, 37.5±8.4 for Si index and 29.5±10 for EI in IDA patients, and 12.8±1.8 for MI, 4.2±0.6 for SI, 59±17.6 for G&K index, 24.8±7.7 for Si index and 13.8±7.3 for EI in BTT patients, which were comparable with the present study.

Si index was found to be more suitable at screening IDA patients with 100% patients being correctly diagnosed to this category and EI correctly identified maximum number of cases in BTT group (48%) in the present study. BI [RDW] performed poorly in both the groups. Several other studies also concluded BI [RDW] to be least valuable to discriminate IDA and BTT [2, 5, 10].

The present study observed high SN and low SP in group A subjects, and low SN and high SP in group B subjects. Such variable results were also observed by Okan *et al.* [10] and Shen *et al.* [14]. In the present study, highest PPV was observed with EI (64.86%) and NPV with index (100%) in group A subjects, while for group B, MI (95.83%) showed highest PPV and EI (64.86%) showed highest NPV. The observations of predictive values were comparable with the reports of Beyan *et al.* [16], Shen *et al.* [14] and Trivedi *et al.* [15]. The values of likelihood ratios were similar with the studies carried out by Pessar *et al.* [29] and Keikhaei *et al.* [30].

YI is a reliable measure for dependability of an index as it takes into account the values of sensitivity as well as specificity, and therefore, by virtue of YI, MI (0.44) and EI (0.44) performed better than the other four indices evaluated for screening MCHC anemia patients. Similar observations were made when accuracy was taken into account, both MI

and E proving to be 72% accurate for screening purposes in the present study.

From the literature reviewed for the present work, the sequence of performance based on the YI for the six selected indices was SI > MI > EI > Si > BI >G&K by Sahli *et al*. [2], G&K > Si > EI > MI > SI > BI by Janel *et al*. [47], G&K > Si > MI > EI > SI > BI by Shen *et al*. [14], G&K > EI >MI > Si > SI = BI by Getta *et al*. [18], G&K > MI > EI > Si > SI > BI by Bhushan *et al*. [26], G&K > MI > Si > EI > SI > BI by Pessar *et al*. [29], G&K > MI > EI > Si > SI > BI by Keikhaei *et al*. [30], G&K > MI > EI > SI > Si > BI by Plengsuree *et al*. [31], BI > G&K > Si > MI > EI > SI by Chandra *et al*. [32], Si > G&K > EI > MI > SI > BI by Tong *et al*. [33] and EI > Si > MI > G&K > SI > BI by Jahangiri *et al*. [34].

Amongst the six indices compared in the present study, MI and EI performed equally well to discriminate IDA and BTT in patients with MCHC anemia based on their values of accuracy and YI. The Our results were consistent with the studies of Rathod *et al*. [8], Ehsani *et al*. [9], Niazi *et al*. [13] and Bhushan *et al*. [26], who suggested the use of either of above mentioned indices for the purposes of screening. The sequence of performance for discriminant indices was MI, EI > SI > Si > G&K > BI [RDW] in the present study, However, Janel *et al*. [7], Shen *et al*. [14], Getta *et al*. [18] and Keikhaei *et al*. [30] reported G&K index to be best for screening MCHC anemia patients, which was discordant to the present study. Our study also reported that BI [RDW] was least acceptable to screen the patients of MCHC anemia and same was stated by Ntaios *et al*. [5], Okan *et al*. [10] and Keikhaei *et al*. [30].

None of the 6 evaluated indices in the present study showed 100% SN or SP, but MI or EI can be used to screen the patients of MCHC anemia as they are able to differentiate the IDA and BTT patients with 72% accuracy, easy to evaluate and require only basic red cell parameters to calculate, which can be readily acquired from automated cell counters available at basic hematology laboratories. The timely screening of these patients can reduce the morbidity and possible delayed treatment of the affected population.

Conclusion

Though none of the indices showed 100% SN or SP, the MI and EI can be reliably used for screening of MCHC anemia patients to categorise them to IDA or BTT in resource limited set ups, so that they can be referred to hospitals with advanced laboratories for confirmatory diagnosis and appropriate treatment. These indices may be embedded to the arithmetic software of automated cell counters by their manufacturers for their fullest potential of clinical usage.

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