Original Research Article

Sehgal index and its comparison with Mentzer's index and Green and King index in assessment of peripheral blood smear with marked anisopoikilocytosis

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ABSTRACT

Background: Mild microcytic hypochromic anaemias due to iron deficiency (IDA) and beta thalassemia trait(β-TT) continue to be a cause of significant burden to the society, particularly in the poorer developing countries. The objective of the present study was to study the RBC based indices in patients of marked anisopoikilocytosis in determining the etiology of it, to standardize few automated red cell parameters, and also objective grading of RBC morphology on peripheral smear and interpreting its utility in indicating a diagnosis. Also, to establish a relation between value of RBC indices with that of degree of anisocytosis.
Methods: A total of 500 patients diagnosed with mild microcytic hypochromic anaemia on complete blood count and peripheral blood film were included in the study. Hb, RBC count, MCV, MCH and RDW obtained from the electronic cell counter were used to calculate discrimination indices by various mathematical formulae. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index (YI) were calculated.
Results: Green and King index demonstrated the lowest sensitivity of 70.51%. Mentzer index demonstrated the highest specificity of 96.80%. The highest and lowest PPV were found for Mentzer index (97.09%) and Sehgal index (92.81%) respectively. Sehgal's index demonstrated the highest NPV of 95.96% and lowest NPV was exhibited by G and K (87.9%). The highest and the lowest values for Youden's index were shown by Sehgal's index (87.82%) and G and K index (68.47%).
Conclusions: Sehgal's index followed by Mentzer index are highly sensitive and reasonably specific in differentiating β-TT from IDA and none of the indices is 100% sensitive and specific. Though HbA2 estimation is the gold standard for diagnosing β-TT, in developing countries, Sehgal index followed by Mentzer et al, index can be used to screen mild microcytic hypochromic anaemia cases to eliminate as many false positive cases as possible to reduce the financial cost.

Keywords: Beta thalassemia trait, Complete blood count, Iron deficiency anaemia, Mentzer index, Sehgal index

INTRODUCTION

Anisocytosis is defined as excessive variation in the size of red blood cells. Clinically, it is quantitatively measured by red cell distribution width (RDW). Elevated RDW can be a reflection of ineffective erythropoiesis, which can be due to nutritional deficiencies, hemoglobinopathies, or underlying bone marrow dysfunction or malignancy. Microcytosis in the setting of an elevated RDW is frequently associated with iron deficiency anaemia and hemoglobinopathies, whereas macrocytosis is associated with reactive erythropoiesis, vitamin B12 and folate

deficiencies, and bone marrow disorders such as myelodysplastic syndromes and leukaemias.[1,2]

Iron deficiency anaemia (IDA) and beta thalassemia trait (B-TT) are the two most frequent disorders presenting clinically with microcytic hypochromic anaemia. Beta-thalassemia trait is a common genetic disorder of hemoglobin and imposes a significant burden on global healthcare. Screening of this disorder is immensely important epidemiologically as it can reduce the future incidence of thalassemia major in newborns. Similarly, IDA is a very frequent finding in developing as well as developed countries. Differentiating mild or moderate IDA from thalassemia trait can be a diagnostic dilemma, as both conditions share many characteristics.[3,4] Accurate and timely detection of these conditions in patients with microcytic anaemia is important as it can provide an indication for supplementing iron to IDA patients, for avoiding unnecessary iron therapy in thalassemia carriers and of course also for preventing severe and lethal forms of thalassemia syndromes in the framework of premarital counselling in high prevalence areas.[5-8]

A definitive differential diagnosis between B-TT and IDA is based on the result of HbA2 electrophoresis, serum iron levels and ferritin levels. However, these investigations are money and time consuming and moreover areas where thalassemia is endemic often have low health care resources and these assays may not be generally available.[9-13]

Past two decades have contributed the several indices such as RBC count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), and red cell distribution width (RDW) to discriminate iron deficiency from thalassemia with its advantages and drawbacks in the practice of clinical hematology. They have been used as a simple and inexpensive tool as these are provided by electronic cell counters to distinguish between BTT and IDA. An ideal discrimination index has high sensitivity and specificity that is it can detect the maximum number of patients with BTT while eliminating patients with IDA.[7,8,13-19]

The reliability of the indices for such discrimination have varying observations. There are studies in the literature which reported the following indices at distinction between beta thalassemia trait and iron deficiency anemia; England and Fraser, red blood cell, Mentzer, Srivastava, Shine and Lal, Bessman, Ricerca, Green and King, Jayabose, Sirdha, M/H ratio, Ehsani and off late youden index, sehgal's index and mato and carvalho index.[4-17]

The sensitivity and specificity of these indices have always remained the crux of debate amongst hematologist to reliably depend on them. It would therefore be interesting to study these indices for comparison and validation at determination of microcytic hypochromic anemia and beta thalassemia trait. Not many studies have dealt on this aspect of microcytic hypochromic anemia in literature search. As these indices and their calculations have variability of the methods, still the objective of each one of them is for discrimination of microcytic hypochromic anemia for purposes of diagnosing and differentiation of iron deficiency anemia, beta thalassemia trait and iron deficiency anemia due to beta thalassemia trait.[20-24]

The present study wants to address the gap of comparisons and indices for better utility of it in clinical hematology practice. Therefore, objectives set in for the present study are as follows.

Aim

To study the RBC based indices in patients of marked anisopoikilocytosis in determining the etiology of it.

Objectives

- To standardize few automated red cell parameters, and also objective grading of RBC morphology on peripheral smear and interpreting its utility in indicating a diagnosis.
- To establish a relation between value of RBC indices with that of degree of anisocytosis.
- To compare the sehgal index (new index) with the existing CBC based indices (DF's) to identify the index having the best combination of sensitivity and specificity to predict anisopoikilocytosis.
- To screen and differentiate IDA and BTT.
- To do comparative evaluation of peripheral blood smear examination and automated red cell indices including RDW, MCV, MCHC, MCHC, fragmented RBC and others, for anisopoikilocytosis in diagnosing anaemia.

METHODS

The cross-sectional study was conducted in the central laboratory, department of pathology, JNMC, DMIMS (DU), Sawangi (Meghe), Wardha, Maharashtra, India from 2017 to 2019.

A total 500 cases (as calculated by formula) were included in this study.

Inclusion criteria

- Participants of the study were the subjects with the age group 3-40 years attending outpatient department of various departments and diagnosed with mild microcytic hypochromic anaemia on complete blood count and peripheral blood film.
- Hb below than the normal value for the subject's age and sex.
- Family members of known cases of beta thalassemia major coming for blood transfusion.

Exclusion criteria

- All patients <3 year of age
- All data with mean cell volume (MCV) >120 fl.

Venous blood samples sent in EDTA vaccutte were tested in automated cell counter as well as peripheral smear examination was done from the slides stained in Romanowsky stains.

All patients with non-neoplastic anemias were screened and selected based on clinical evaluation and hemoglobin values.

Hematological studies included complete blood count with Hb concentration, MCV, MCH and RDW by automation and comparing it to the general blood picture.

All peripheral blood films were prepared manually and were stained by a single trained person to minimize variation in smear spreading and staining due to interpersonal differences in technique. Two practicing hematologists and one second year medical junior resident examined each peripheral blood film independently. Each peripheral blood film was visually graded for RBC morphology following standard guidelines. High performance liquid chromatography technique was used for determination of HbA2. Several simple screening indices which have been developed so far for differentiating between thalassemia trait and IDA are as follows (Table 1) and were analysed and compared

for their values. In this study, authors have compared the results of Sehgal's index with Mentzer's and Green and King index as authors want to compare the efficacy of newly discovered Sehgals index with the oldest one i.e., Mentzer index and the most widely used one i.e., Green and King index.

Statistical analysis

Statistical tests will be carried out for specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and Youden's index by standard formulae for all the three indices.

The significant erroneous diagnosis will be evaluated by peripheral smear reporting and by statistical tests if required. p value less than 0.05 were considered significant.

RESULTS

A total of 500 cases with anisopoikilocytosis who met the inclusion criteria of the study were recruited, of which 320 (64%) were females and 180 (36%) were males. Maximum number of patients belonged to 5-20 years of age (n=275).

Majority, of the patients in the study had no obvious complaints related to anemia (60%) but weakness (35%) followed by fatigue (5%) were the next common complaints received.

Table 1: Various indices with their cut-off value.

Discriminant index	Year of discovery	Calculation	Cut-off value
MI (Mentzer's index)	1973	MCV/RBC	13
E and F (England and fraser)	1973	MCV-RBC-(5×Hb)-3.4	0
SI (Srivastava index)	1973	MCH/RBC	3.8
S and L (shine and lal)	1977	MCV×2×MCH/100	1.58
RDWI (Red cell distribution width index)	1987	MCV×RDW/RBC	220
RI (Ricera index)	1987	RDW/RBC	4.4
G and K (green and king)	1989	(MCV×2×RDW)/(HB x 100)	65
Sirdah index	2007	MCV-(5×Hb)-RBC-3.4	27
Ehsani et al index	2009	MCV-(10×RBC)	15
Sehgal index	2016	MCV×MCV/RBC	972

Table 2: Mean values and standard deviation of various haematological parameters.

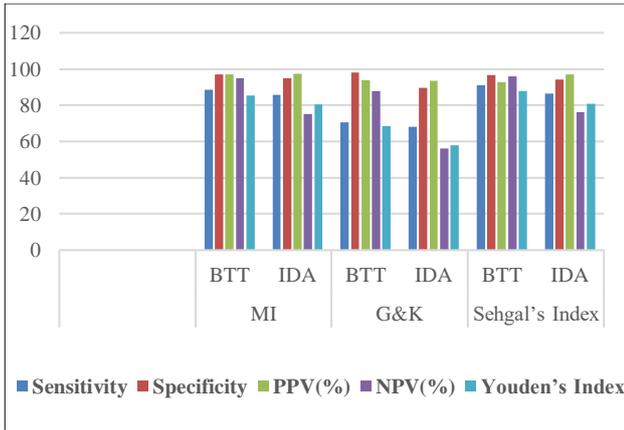| Parameter	Beta thalassemia		IDA		p-value
	Range	Mean±SD	Range	Mean±SD	
Hb	9-11.5	10.7±0.8	8.6-11.3	9.9±1.43	<0.001
RBC	4.6-6.4	5.79±0.76	3.7-5.3	4.77±0.53	<0.001
MCV	56.4-71.1	63.6±5.49	54.2-78.4	69.4±5.16	<0.001
MCH	18.4±2.15	18.4±2.15	14.2-26.7	20.67±1.34	<0.001
RDW	14-22.9	15.9±1.62	12.7±27.1	17.2±2.96	<0.003

Figure 1: Comparison between the discriminant indices for detecting BTT.

On the basis of HbA2 levels,156 patients having HbA2 more than 3.5% were grouped into B-TT group. Rest 344 patients having HbA2 less than 3.5% were included in the IDA group. The mean values for Hb in B-TT group were 10.7±0.8 and those in the IDA group were 9.9±1.43 as shown in Table 2.

All parameters for IDA and BBT were significantly different as determined by an independent t-test analysis. The number and percent of IDA and BBT identified by each of the 3 indices used in this study is shown in Table 3. The percent of IDA and BBT identified by discriminant functions was based on the number identified by the discriminant function/the total number of IDA and BBT. Mentzer index and sehgal index detected more than 85% of IDA and only sehgal index could detect more than 90% of BTT.

Table 3: Number of cases correctly identified by 3 discriminant indices.

Discriminant function	Cut off values	IDA(n=344)	BTT(n=156)
Mentzer's index	IDA >13, BTT <13	295 (85.75%)	138 (88.4%)
Green and king index	IDA >72, BTT <72	234 (68%)	110 (70%)
Sehgal's index	IDA >972, BTT <972	298 (86.62%)	142 (91%)

Table 4: Sensitivity, specificity, positive and negative predictive value, and Youden's index of each discriminant function used in differentiation between IDA and BTT.

Discriminant function		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
MI	BTT	88.4%	97.09v	97.09%	94.8%	85.49
	IDA	85.75%	94.87%	97.35%	75.1%	80.62
G and K	BTT	70.51%	97.96%	94.01%	87.9%	68.47
	IDA	68%	89.74%	93.6%	56%	57.74
Sehgal's index	BTT	91.02%	96.80%	92.81%	95.96%	87.82
	IDA	86.62%	94.23%	97.06%	76.16%	80.85

The effectiveness of each discriminant function to distinguish IDA from BTT were analysed for the following parameters - sensitivity, specificity, positive and negative predictive values and the Youden index (Figure 1).

As is evident from the results shown in Table 4, none of the indices showed 100% efficiency in recognizing beta thalassemia trait. Green and King index demonstrated the lowest sensitivity of 70.51% which means it missed about 29.49% of B-TT cases. Mentzer index demonstrated the highest specificity of 96.80%. The highest and lowest PPV were found for Mentzer index (97.09%) and Sehgal index (92.81%) respectively. Sehgal's index demonstrated the highest NPV of 95.96% and lowest NPV was exhibited by G and K (87.9%).

The highest and the lowest values for Youden's index were shown by Sehgal's index (87.82%) and G and K

index (68.47%). None of the indices was completely sensitive and specific in differentiating between BTT and IDA.

DISCUSSION

In the Indian population, IDA and BTT are common causes of microcytic hypochromic blood picture.[3-6] The real danger of non-diagnosis and misdiagnosis of carriers of thalassemia trait is potential homozygous offspring. Appropriate screening, detection of patients, and counseling of couples at risk are the most important measures for the reduction of morbidity and mortality.[7] 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.

Since 1973, several indices have been introduced in an attempt to distinguish these two conditions in a cheaper and easier way. According to the original published papers by the authors of these indices, their sensitivity in the detection of β-TT from IDA is approximately 100%.[1-7] However later studies failed to confirm these results and estimated these indices' sensitivity to be between 61-91%.[8-20] These indices incorporate MCV, MCH, RBC Count, RDW and HB in various combination.

Table 2 shows that there were significant differences between the hematological parameters of β-TT and IDA but these did not reflect in the indices' reliability in differential diagnosis of β-TT and IDA. In the present study, the Sehgal index showed a reasonably high sensitivity, specificity and good Youden's index of 91.02%, 96.80% and 87.82% respectively. This was followed by Mentzer index which showed sensitivity, specificity and Youden's index of 88.4%, 97.09% and 85.49% respectively. Ehsani et al, showed that the best discrimination index according to youden's criteria was the Mentzer index (90.1%), followed by the Ehsani et al, index (85.5%). In their study, the Mentzer and Ehsani et al, indices were able to correctly diagnose 94.7% and 92.9% of cases, respectively.[16]

In the present study, the green and king index demonstrated a sensitivity, specificity and Youden's index of 70.51%, 97.96% and 68.47% respectively. The findings are comparable with the results obtained by Telmissani et al, who found these to be 85.7%, 79.4% and 65.0% respectively.[14]

In 2007, Ntaios et al, reported that the green and king index was the most reliable index, as it had the highest sensitivity (75.06%), efficiency (80.12%), and Youden's index (70.86%) for detecting β-TT.[6] A similar result for the green and king index (Youden's index, 80.9%) was found by Urrechaga et al.[1] However in the present study, though the sensitivity was 70.51%; it showed a low Youden's index of 68.47%.

Madan et al studied 658 (126: BTT with IDA, 337: pure BTT, 195: IDA, 40: normal) cases of HPLC.[24] They found Meltzer's index <14 to have a BTT detection rate of 88.7% which is comparable to the current study (91.02%). The difference between these studies and present study could possibly due to the high incidence of concomitant IDA in this study subset. So, in the present study, according to the Youden's index which is a measure of high performance of the discrimination index, following rankings of various discrimination indices were obtained. Sehgal>Mentzer>Green and King.

As seen above, none of the formulas and parameters can be 100% sensitive and specific, and various studies have shown different results showing one formula to be better than the other. There are many reasons for the discrepancies with the main being the difference in the population subsets evaluated, the prevalence of nutritional deficiency anemia in the population studied and different yardsticks used for evaluation of formulas. In addition, most studies have used HbA2 levels for identification of BTT and its well-known that there are silent BTT cases with normal HbA2 levels.

In this study, Sehgal index and Mentzer's index <14 had the highest Youden index. A high Youden index indicates a good combination of sensitivity and specificity implying its utility for screening of BTT in a tertiary hospital setup. The Sehgal index (MCV/MCV/RBC) by squaring the MCV value essentially utilizes the power of MCV to separate out BTT cases more effectively. Instead of using a two-step screening process of first identifying a case with low MCV and then applying the Mentzers index (MCV/RBC); the Sehgal index converts it into a one-step screening process.

This study 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.

CONCLUSION

The above data clearly states that Sehgal's index followed by Mentzer index are highly sensitive and reasonably specific in differentiating β-TT from IDA but none of the indices is 100% sensitive and specific. Although estimation of HbA2 remains the gold standard for diagnosing a case of β-TT, a practical approach particularly in resource limited settings can be to screen mild microcytic hypochromic cases with these two indices and try to eliminate the false positive cases as much as possible to reduce the financial burden. Also, Sehgal index can be implemented as a validated flag rule in CBC analyzer middleware program for identifying suspected cases of β-TT. This will drastically improve the detection rate for β-TT cases in our routine practice. Using one of the above-mentioned indices as a "validated flag" in the current generation of CBC analyzers will be definitely helpful for tertiary care centers with a high incidence of β-TT.

Discrimination indices depending on automated cell counter-based parameters are rapid, reliable, and easy tools to suspect a case of β-TT, and send the subject for further HPLC evaluation.

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