ORIGINAL ARTICLE

Application of Hematological Indices for the Differential Diagnosis of Beta Thalassemia Trait and Iron Deficiency Anemia

Sajid Abbas[1], Shaista Khurrum[2], Tahira Zamir[3]

ABSTRACT

Objective: To apply various hematological indices for the differential diagnosis of ß-Thalassemia trait (ß-TT) and iron deficiency anemia (IDA).

Methodology: This retrospective descriptive study was conducted at Dar-ul-Sehat Hospital, Gulistan -e- Johar, Karachi. We retrospectively analyzed complete blood count (CBC) of 2480 patients, who came to the OPD for various problems during the year 2014 from January to December so as to identify hypochromic microcytic patients. Mentzer's index (MI), Shine and Lal index (S and L index) and Ehsani's formula were applied on the CBC report of identified microcytic hypochromic patients.

Results: It was found that among a total of 2840 patients, 385 (13.55%) patients were suffering from hypochromic microcytic anemia identified on their CBC report. These included 44 males (6.74%), 300 females (33.33%) and 41 (14.48%) children. Application of Mentzer's index (MI), Shine and Lal index (S and L index) and Ehsani's formula screened the hypochromic microcytic patients into patients suffering from ß-Thalassemia trait and Iron deficiency Anemia.

Conclusion: Application of hematological indices can be taken as the most useful method for differentiating ß-TT from IDA by simply considering CBC report.

Keywords: Hematological Indices, Iron deficiency anemia, ß-thalassemia trait, Mentzer's index, Shine and Lal Index, Ehsani's Formula

INTRODUCTION:

Anemia resulting from lack of sufficient iron to synthesize hemoglobin is the most common hematological disease in Pakistan. The World Health Organization (WHO) estimates that worldwide, 42% of pregnant women, 30% of non-pregnant women (aged 15-50 years), 47% of preschool children (aged 0-5 years) and 12.7% of men older than 15 years are anemic.[1] The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and ß-thalassemia trait (ßTT).[2] Iron deficiency anemia is most commonly associated with inflammatory bowel diseases, pregnancy, menstruation, lactation, surgery, physical trauma, vegetarians and children who drink more than 16-24 ounces a day of cow milk.[3] Without enough iron, body cannot produce enough hemoglobin in red blood cells that enables them to carry oxygen.[4] On the other hand, ß-thalassemia trait is a hereditary microcytic hypochromic anemia characterized by the production of abnormal hemoglobin. Approximately, 1.5% of the world's population is a carrier for ß-thalassemia trait. It is a carrier state in which only one allele is mutated. Individuals with the ß-thalassemia trait are usually asymptomatic and may be unaware of their carrier state unless diagnosed by investigations.[5] It is the most common type of hemoglobinopathy transmitted genetically. According to one study, 8 million people are carriers of thalassemia in Pakistan.[6]

It has been observed that the CBC (Complete Blood Count) results of both these types of microcytic anemia usually overlap. Traditional approach followed by most general practitioners is a trial of iron treatment which imposes a significant burden on global healthcare.[7] A definitive differential diagnosis between ß-TT and IDA is based on the result of HbA_2 electrophoresis, serum iron levels, and a ferritin calculation.[8] Many formulas have been proposed by researchers having the ability to differentiate iron deficiency anemia from ß-thalassemia trait (Table-1). These formulas include a minimum of two CBC parameters in various combinations. The only purpose of using CBC indices for the discrimination of microcytic hypochromic anemia is to reduce unnecessary investigation cost of iron therapy and problems of iron overload.[9] It is simple and inexpensive tool to give a clue to differentiate both diseases and identify patients who require follow up and counseling.[10] In outpatient department (OPD) of one local hospital especially in Pakistan where resources are limited, the exclusion of thalassemia minor could be achieved mathematically using the CBC indices.[11] In this study, Mentzer's index (MI), Shine and Lal index (S and L index) and Ehsani formula were applied on the CBC report of these identified microcytic hypochromic patients.[12]

METHODOLOGY:

This retrospective descriptive study was conducted at

✉ **Dr. Sajid Abbas**
Professor and Head, Department of Medicine
Bahria University Medical and Dental College
Karachi.
✉ **Dr. Shaista Khurrum**
Lecturer, Department of Pathology
Bahria University Medical and Dental College
Karachi.
✉ **Dr. Tahira Zamir**
Assistant Professor, Department of Pharmacology
Karachi Institute of Medical Sciences
Karachi.
Received: 05-02-2017
Revised: 12-02-2017
Accepted: 26-03-2017

Dar-ul-Sehat Hospital, Gulistan-e- Johar, Karachi for a period of 1 year from January 2014-December 2014. We retrospectively analyzed Blood CBC of 2480 patients who came to the OPD for various problems. None of them had received a transfusion or had an acute bleeding episode in the previous month. Patients included were males, females and children (less than 14 years of age). Blood samples were collected in EDTA anticoagulant tubes. Automated cell counter was used for CBC test at Dar-ul-Sehat Laboratory for hematological testing. Patients of Microcytic Hypochromic anemia were identified after analyzing CBC reports using WHO standard cutoff values.[13] Hematological indices were then calculated for each case so as to make differential diagnosis of patients either as iron deficiency anemia or ß-Thalassemia Trait.

RESULTS:

Among 2480 patients who reported to the OPD, 900 patients were males, 1200 females and 380 children. Their CBC report was analyzed retrospectively for identification of anemia. It was found that 385 (13.55%) patients were suffering from hypochromic microcytic anemia. These included 44 males (6.74%), 300 females (33.33%) and 41 (14.48%) children (Table-2). We applied Mentzer's index (MI), Shine and Lal index (S and L index) and Ehsani formula on the CBC report of these identified microcytic hypochromic patients.

Table: 1

Hematological Indices and Mathematical Formula used to differentiate between IDA and ß-TT[14]

Hematological index	**Formula**
Mentzer index (MI)	MCV/RBC
RDWI	MCV × RDW/RBC
Shine and Lal (S and L)	MCV × MCV × MCH/100
Srivastava	MCH/RBC
Green and King (G and K)	MCV × MCV × RDW/Hb × 100
Sirdah	MCV - RBC - (3 × Hb)
Ehsani	MCV - (10 × RBC)
England and Fraser (E and F)	MCV - (5 × Hb) - RBC - 3.4
Ricerca	RDW/RBC
MDHL	(MCH/MCV) × RBC
MCHD	MCH/MCV

MDHL index: Mean Density of Hb/Liter of blood; MCHD index: Mean cell Hb Density

Table: 2

Data of patients included for CBC report

S. No	Number of Patients (N=2840)	Age Range (Years)	Normal	Microcytic Hypochromic Anemia	Percentage (%)
1	Male 900	15-60	652	44	6.74%
2	Female 1200	15-60	900	300	33.33%
3	Children 380	Less than 14	283	41	14.48%

According to Mentzer's index, 41 (93%) men presented with IDA, 3 men (7%) presented with ß-TT (7%), 293 females (97.66%) presented with IDA, 7 (2.33%) females presented with ß - TT, 37 (90.24%) children presented with IDA, 4 children (9.75%) presented with ß- TT

Table: 3
Differential Diagnosis of Patients based on Mentzer's Index (MI)

Gender	IDD	Percentage (%)	ß-TT	Percentage (%)
Male	41	93 %	3	7%
Female	293	97.66%	7	2.33%
Children	37	90.24%	4	9.75%

According to Shine and Lal index (S and L index), 39 (88.63%) men presented with IDA, 5 (11.36 %) men presented with ß -TT, 292 (97.66%) females presented with IDA, 8 (2.66%) females presented with ß-TT. Children who had IDA were 38 (92.68%) and those with possible ß-TT were 3 (7.31%)

Table: 4
Differential Diagnosis of Patients based on Shine and Lal (S and L) index

Gender	IDD	Percentage (%)	ß-TT	Percentage (%)
Male	39	88.63 %	5	11.36%
Female	292	97.33%	8	2.66%
Children	38	92.68%	3	7.31%

According to Ehsani's formula, men presented with IDD were 41(93.1%), presented with ß-TT were 3 (6.8%), women who suffered from IDA were 296 (98.6%), had ß-TT were 4(1.3%), children who were found to have IDA were 39(95.1%) and possibly had ß-TT were 2 (4.8%).

Table: 5
Differential Diagnosis of Patients based on Ehsani's formula

Gender	IDD	Percentage (%)	ß-TT	Percentage (%)
Male	41	93.1 %	3	6.8%
Female	296	98.6%	4	1.3%
Children	39	95.1%	2	4.8%

DISCUSSION:

Differentiation of iron deficiency anemia from thalassemia minor is clinically significant because each disease has entirely different etiological factors, management, genetic counseling and dietary plan. In addition to genetic counseling, in case of thalassemia carriers, iron therapy is warranted because the thalassemia heterozygote should not be given iron to normalize MCV.[15] Health experts claim that approximately 5000 children are diagnosed with Thalassemia major every year in Pakistan. On the other hand iron deficiency anemia is more common in children and adult women especially during reproductive time period. Diagnosis of ß-Thalassemia trait is established by the presence of characteristic RBC microcytosis and elevated levels of serum iron (SI), transferrin saturation (TS) and ferritin with increased levels of HbA2.[16] Decreased levels of SI, TS and ferritin with increased levels of SBC are the main diagnostic criteria for IDA.[17] A variety of formulae and indices have been proposed to facilitate the screening procedure of iron deficiency anemia and ß-thalassemia trait like Mentzer's index, England and Frasen, Shine and Lal, and Ehsan and Shrivastve formulae.[18] These formula can be taken as the most reliable and predictive for differential diagnosis of iron deficiency anemia from ß-Thalassemia trait by simply considering CBC. Moreover, these can be helpful in preventing the fatal disease, ß-Thalassemia major.[19] Different RBC indices can correctly identify 61-91% of patients with microcytic anemia.[20]

In our study, we successfully applied three formulae on large number of patients coming to Dar-ul-Sehat hospital and diagnosed to have microcytic anemia so as to differentiate them into IDA and ß-TT.

Results of our study indicated that 54.55% of the population including male, female and children were suffering from microcytic hypochromic anemia and application of these various formulae on the CBC indices of these patients successfully identified patients of IDA and ß-TT.

In 2009, Ehsani et al showed that the best discrimination index according to Youden's criteria was the Mentzer index (90.1%), followed by Ehsani index (85.5%).[21]

According to our results, percentage of patients diagnosed varied with various formulae. None of the formula was found superior to other in terms of percentage.

In 2007, Saud et al in their research work applied nine formulae of RBC indices on a population of 153 confirmed cases of microcytic anemia and measured validity using Youden's index. They found that the E and F index had the highest Youden's index (98.2%), specificity and sensitivity in correctly differentiating IDA and Thalassemia minor patients.[22] Fakher and Bijan[23] also applied various formulae on 323 confirmed cases of microcytic anemia and showed that the S and L index had the highest Youden's index (89%) in patients younger than 10 years of age while RDW and RBC indices have the highest Youden's index 93% and 90% respectively in patients older than 10 years of age. Nitaios et al used six indices for differentiation between 373 patients of microcytic anemia and found that G and K index had the highest reliability, followed by E and F, RBC Count, MI and RDWI. RDW completely failed to differentiate between IDA and TT.[24]

Beyan et al in 2007 identified 66 cases of ß-TT and 45 cases of IDA. Patients and groups were evaluated according to RBC, MI, S & L, E & F, S I, G & K and Ricevca index. They concluded that none of these formulae is superior in differentiating IDA from ß-TT and total body iron and HB A2 level should be obtained for accurate differential diagnosis.[25] The results of our study were in accordance to the results of Beyan et al.

All formulae used should have a good sensitivity score so as to detect maximum number of patients and these should be able to eliminate as many other patients as possible to avoid false positive results. The formulae we have used have high sensitivity and specificity as confirmed by various researchers.[26]

CONCLUSION:

According to our study, these hematological indices can be useful for differential diagnosis of ß-TT from IDA by simply considering CBC report. This can be helpful in preventing ß-thalassemia major disease and iron overload in future.

REFERENCES:

1. World Health Organization. Geneva, Switzerland; Guideline: Intermittent Iron Supplementation for Preschool and School Age Children. e-Library of Evidence for Nutrition Actions (eLENA). World Health Organization; 2011
2. Khambalia AZ, Aimone AM, Zlotkin SH. Burden of anemia among indigenous populations Nutr Rev. 2011; 69 (12): 693-719
3. Matos JF, Dusse LM, Stubbert RVB, Ferreira MR, Coura-Vital W, Ferreira MR, et al. Comparison of discriminative indices for iron deficiency anemia and beta thalassemia trait in a Brazilian population. Hematology, 2013; 18:169-174
4. Nalbantoglu B, Guzel S, Buyukyalcin V, Donma MM, Guzel EC, Nalbantoglu, et al. Indices used in differentiation of thalassemia trait from iron deficiency anemia in pediatric population are they reliable? Pediatr Hematol Oncol, 2012; 29:472-478
5. Charles A. Linker. Blood: Current med diagnosis and treatment: Blood-Anemias. Lange Med Pub 2004; 43:462-463
6. Ansari SH, Shamsi TS, Ashraf M, Bohray M, Farzana T, Khan MT, et al. Molecular epidemiology of B-thalassemia in Pakistan. Far reaching implication. Int J Mol Epidemiol Genet 2011; 2: 403-8
7. Batebi A, Pourreza A, Esmailian R. Discrimination of beta-thalassaemia minor and iron deficiency anemia by screening test for red blood cell indices.Turk J Med Sci 2012; 42: 275-280
8. Martin A, Thompson AA. Thalassemia. Pediatr clin North Am, 2013; 60: 1383-1391
9. Mentzer WC. Differentiation of iron deficiency anemia from thalassemia trait. Lancet 1973; 1: 882
10. Usman M, Moinuddin M, Ahmed SA. "Role of iron deficiency anemia in the propagation of beta thalassemia gene." Korean Journal of Hematology 2011; 46(1): 41-44
11. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. "Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the B. thalassemia minor from iron deficiency in Palestinian Population." International journal of laboratory Hematology, 2008; 30(4): 324-330
12. Urrechaga E, Borque L, Escanero JF. The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and ß-thalassemia screening. American Journal of Clinical Pathology 2011; 135(3): 374–379
13. Khusun H, Yip R, Schultink W, Dillon DHS. World Health Organization Hemoglobin Cut-Off Points for the Detection of Anemia Are Valid for an Indonesian Population.The American Society for Nutritional Sciences 1999
14. Vehapoglu Y, Ozgurham 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; 2014, Article ID 576738, 7 pages http://dx.doi.org/10.1155/2014/576738
15. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. Turk J Hematol. 2009; 26(3):138-45
16. Penman BS, Gupta S, Weathered DJ. Epistasis and the sensitivity of phenotype screens for beta thalassemia. Br J Haematol 2015; 169(1):117-128
17. Rathod DA, Kaur A, Patel V, Ptel K, Kabrawala R, Patel M, et al. Usefulness of cell counter-based parameters and formulas in detection of ß-thalassemia trait in areas of high prevalence. American Journal of Clinical Pathology 2007; 128(4):585–589
18. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia. Pediatr Int 2002; 44:612-6

19. Huang TC, Wu YY, Chen YG, Lei SW, Wu SC, Ye RH, et al.. Discrimination index of microcytic anemia in young soldiers in a single institutional analysis. Plos One 2015; 10(2): e0114061
20. Miri-Moghaddam E Sargolzaie N. Cut off Determination of Discrimination Indices in Differential Diagnosis between Iron Deficiency Anemia and ß-Thalassemia. Int J Hematol Oncol Stem Cell Res. 2014;8(2): 27–32
21. 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 2009; 12(5): 473-5
22. Suad MA, Anwar M Al-A, Doa'a A. Validity assessment of nine discriminant functions used for the differentiation between iron deficiency anemia and thalassemia minor. J Trop Pediatr 2007; 53: 93-7
23. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta thalassemia trait. Turk J Hematol 2009; 26:138-45
24. Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for beta-thalassemic trait. Ann Hematol 2007; 86: 487-91
25. Beyan C, Kaptan K, Ifran A. Predictive value of discrimination indices in differential diagnosis of iron deficiency anemia and beta-thalassemia trait. Eur J Haematol 2007; 78: 524-6
26. Garg S, Srivastava A, Singh S, Jaiswal R, Singh YK. Role of hematological Indices in the screening of ß-thalassemia Minor (trait) and iron deficiency Anemia. American research journal of Hematology 2016; Volume 2016: 1-5