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Original Article

Discrimination Indices for Diagnosis of Beta(β) Thalassemia Trait

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Abstract

Background: To determine the reliability of hematological indices and derived formulas in diagnosing beta thalassemia trait.

Methods: In this observational cohort study, patients diagnosed as beta thalassaemia minor were included. All hematological indices were recorded.Haemoglobin electrophoresis was performed using capillarys 2 flex piercing system. An HbA2 value > 3.5% was considered as a cut-off point for beta-thalassemia trait. Fourteen haematological indices were then applied according to the formulae and cut off values. Percentage of positive cases accurately identified along with the mean, median and mode were calculated for each discrimination index.

Results: The study cohort constituted of total 493 patients, out of which 246 (49.9%) were male and 247 (50.1%) were females. The mean age of all the patients was 20.34 ±12.835, with range of 6-50 years. The mean haemoglobin level of all patients was 10.82±1.64 and ranged between 4.60 and 16.30. A red blood cell count more than 4.9 X 10 ^{12}/l and a MCH less that 25 pg were the most consistent findings. Srivastava index was more near to predicting beta thalassaemia minor .

Conclusion: Red cell indices and discrimination factors have a potential utility in screening for β thalassaemia trait , keeping in view their sensitivity and specificity

Key Words : Discrimination indices, Beta(β) thalassaemia trait,

Introduction

Beta (β) thalassaemia is considered as the world's most wide spread genetic disease. In Pakistan the prevalence of its carrier rate varies from 5 to 7% in different areas. The disease's severity , with high morbidity and mortality justifies the implementation of preventive strategies . Early screening of carriers and counseling is essential for prevention of β – thalassaemia. [1,2] The confirmation of β – thalassaemia carriers is by detecting high HbA_2 levels, on haemoglobin electrophoresis, or in rare cases by mutation analysis (e.g., cap site mutations). Red cell indices on automated blood counters and then different discriminating formulae, based on red cell indices, can significantly improve the selection of cases for further evaluation . [3-15] The purpose of using red blood cell indices to discriminate is to detect subjects who have a high probability of requiring appropriate follow up and to reduce unnecessary investigative costs. Various formulae have been proposed according to the index of red blood cells. All these formulae have been tested with different cut off values and then to evaluate their sensitivity and specificity.The ideal discriminating index will be the one which will have high sensitivity (to detect maximum number of β – thalassaemia carriers) and high specificity (eliminating iron deficiency patients). [4, 8, 16-20]

B- thalassaemia minor and iron deficiency anaemia are the most commonly encountered hypochromic microcytic anaemias. The differential diagnosis between iron deficiency anaemia and β – thalassaemia minor in an important concern for every physician to avoid unnecessary iron therapy and false diagnosis of beta thalassaemia minor, especially in pre- marriage counselling , towards prevention of β – thalassaemia major baby birth and minimization of expenses . Prevention of β – thalassaemia major is one of the most important programs of health system especially in countries with high β – thalassaemia gene prevalence . Screening of carriers, especially extended family screening in a family with a case of beta thalassaemia major (index case) and counselling at risk couples are the most successful approaches in reduction of new cases of β – thalassaemia major .

Beta thalassaemia minor an iron deficiency anaemia have a similar pattern of hypochromic microcytic anaemia. Definitive methods for differential diagnosis between β – thalassaemia minor and iron deficiency anaemia include haemoglobin electrophoresis and DNA mutation analysis. At mass level, if these

facilities are not available then red blood cell indices and morphological examination of red cells morpohology , can give a road map how to proceed further. Red cell indices and then different discriminating factors can help to make a safe guess. Peripheral blood film examination can also give a clue. Microcytic hypochromic red cells morphology with anisocytosis proportionate to degree of anaemia and presence of pencil shape cells indicates iron deficiency , while uniformly microcytic hypochromic picture with target cells and very minimal anisocytosis is indicative of β – thalassaemia minor . [6,21]

Subjects and Methods

This was on observational retrospective cohort study, conducted at Punjab Thalassaemia Prevention Programme (PTPP) laboratories , at Holy Family hospital, Rawalpindi. All the patients diagnosed as beta thalassaemia minor form April to June 2015 were included. Ethical approval for the study was sought from institutional research board, Rawalpindi Medical College. Details of patients'age and sex were recorded. All hematological indices were recorded.The maximum time elapsed from blood sample collection till testing was 1 day.

Table 1: discrimination indices for diagnosis of beta thalassemia trait.

Discrimination index	Formula applied	Cut-off value for suspecting beta thalassemia trait
Mentzer index (MI).[18]	MCV/RBC	< 13
Shine & Lal index. [22]	MCV×MCV×MCH ×0.01	< 1530
England & Fraser Index. [23]	MCV- RBC-5×Hb-8.4	< 0
Srivastava index. [16]	MCH/RBC	< 3.8
Green & King Index. [23]	MCV×MCV×RDW/ Hb×100	< 65
RBC distribution width index(RDWI) [24]	MCV×RDW/RBC	< 220
Ricerca(R) index . [25]	RDW/RBC	< 3.3
Keikhaei index. [26]	Hb×RDW×100/RBC ×RBC×MCHC	< 21
Mean Density of Hb/ litre of blood (MDHL). [27]	MCH/MCV×RBC	>1.63
Mean Cell Hb Density(MCHD) . [27]	MCH/MCV	>0.3045
Ehsani et al index. [19]	MCV - 10× RBC	< 15
Sirdah et al index. [4]	MCV-RBC-3×Hb	< 27
Hisham index . [13]	MCH×RDW/RBC	< 67
Hameed index . [13]	(MCH×Hct×RDW)/(RBC×Hb)	< 220

None of the patients had received blood transfusion in the last 3 – 4 months. For each patient a 3 mL intravenous blood sample was collected in EDTA-containing blood collection tubes. All samples were processed for haematological indices using a fully automated blood cell counter. Hemoglobin electrophoresis was performed on all the samples within 1 day using .Capillary's 2 Flex Piercing system in the presence of controls and normal and abnormal haemoglobin curves noted. An HbA2 value > 3.5% was considered as a cut-off point for beta-thalassemia trait. Fourteen Haematological indices were then applied according to the formulae and cut off values,compared with that of Haemoglobin electrophoresis (Table 1). The proportion of Beta Thalassemia carriers identified correctly by each of these indices was computed. Percentage of positive cases accurately identified along with the mean, median and mode were calculated for each discrimination index.

Results

The study cohort constituted of total 493 patients, out of which 246 (49.9%) were male and 247 (50.1%) were females. The mean age of all the patients was 20.34 ±12.835, with range of 6-50 years. The mean hemoglobin level was 10.52±1.71.

Table 2: Red cell characteristics in β – thalassaemia minor

	Mean ± SD	Range
Hb (g/dl)	10.53± 1.71	8.1- 14.0
RBC count (X 10^6/ul)	5.70 ± 0.82	4.95-7.28
MCV (fl)	58.21± 3.86	48.30-73.80
MCH (g/dl)	20.1 ± 2.1	16.82- 25.2
HbA2 (%)	4.90± 0.61	3.6- 7.8

Table 3: Summary of hematological indices in our study population.

Index	Cut off value	Positive	Negative	Mean	Median	Mode	Standard deviation
Mentzer group	<13	386, (78.3%)	106, (21.5%)	11.68	17.36	9.51	2.48
Shine And Lal group	<1530	485, (98.0 %)	8, (1.6%)	819.86	789.83	467.17	394.75
England And Fraser	< 0	356, (72.2%)	137, (27.8%)	-4.6	-4.3	-7.3	-8.75
Srivastava	≤ 4	493, (98.5%	0, (0%)	3.68	3.41	2.65	1.65
Green And King	< 65	267, (54.2%)	226, (45.8%)	67.15	63.09	59.59	18.71
RDW index	< 220	353, (71.6%)	140, (28.4%)	205.29	192.45	167.72	55.52
Ricerca index	< 3.3	323, (65.5%)	166, (33.7%)	3.24	3.05	2.84	0.86
Keikhaei index	< 21	317, (64.3%)	174, (35.3%)	20.56	19.29	16.74	5.6
MDHL	> 1.63	304, (61.7%)	181, (36.7%)	1.74	1.69	1.35	0.79
MCHD	> 0.3045	240, (48.7%)	253, (51.3%)	0.31	0.3	0.33	0.13
Ehsani et al index	< 15	382, (77.5%)	111, (22.5%)	7.71	7.8	-0.6	10.38
Sirdah et al index	< 27	302, (61.3%)	191, (38.7%)	25.45	25.08	22.1	6.75
Hisham index	< 67	348, (70.6%)	145, (29.4%)	64.58	58.74	47.35	29.17
Hameed Index	< 220	353, (71.6%)	140, (28.4%)	208.32	192.52	168.73	87.67

Raised RBC count (≥ 4.9 X 10^6 / ul)and a decreased MCH (≤ 25.2 g/dl) were the consistent findings

(Table 2) In our study population, Srivastava index was most successful in correctly predicting beta thalassemia trait in patients (correctly predicting in 98% patients), while other indices such as MCHD and Green And King index did not have a very high predictive accuracy, (predicting correctly in 48.7% and 54.2% people respectively) (Table 3).

Discussion

β – thalassaemia minor has a prevalence varying from 5-7% in different areas of Pakistan. Early screening and counselling is essential for prevention of β – thalassaemia major.[1,2] In clinical practice , it can be assumed that red blood cell indices should be sufficient to raise suspicion of β – thalassaemia minor and thereby leading to performance of further evaluation , in required cases. Despite this logical rationale , most β – thalassaemia carriers are detected randomly or during mass screening or when a new case emerged in a family . This backdrop situation as well as the burden of β – thalassaemia major patients for health services of a country have compelled many countries to develop screening programs for β – thalassaemia prevention. Although the prevention programs in many countries have succeeded to lower the prevalence of giving birth to affective children, yet they have financial and operative constraints , especially in under or developing countries. [28] In Pakistan with this backdrop Punjab Thalassaemia Prevention Program (PTPP) was launched with an objective to provide free facility of carriers detection in families (extended family screening) where there is a case of β – thalassaemia major (Index case) . This program also offers free prenatal diagnosis services to couples where an unchecked pregnancy may lead to birth of β – thalassaemia major child . Present study comprises data of β – thalassaemia carriers diagnosed at PTPP laboratory at Holy Family Hospital , Rawalpindi

In clinical practice it is required to differentiate iron deficiency anaemia and β – thalassaemia minor . In order to reduce the cost, time and complicated procedures for their discrimination, various red blood cell indices and formulae have been used. The most β – thalassaemia minor cases are asymptomatic and without specialized tests may be missed or sometimes misdiagnosed as iron deficiency anaemia. [29] Lack of access to specialized laboratory facilities across the country necessitates to screen out carriers at the first instance by first line tests. To a greater extent this objective can be achieved by carefully analyzing red blood cell indices. [6]

The spectrum of β – thalassaemia mutations in each population can affect on various RBC indices , therefore it is suggested to determine cut off value for every formula in different population. [6] A high specificity and minimal false negative results are required to confirm the formulas' reliability. [3] In a reliable formula , a negative predictive value higher than 99% is enough to recognize a formula reliable for daily use . A program that intends to become safe for mass population screening should miss as few false negative samples as possible. Sensitivity and specificity of these indices and formulae have been evaluated in several studies. Varied results are likely to be due to different genetic mutations. It is required to establish cut off values for these discriminating factors in accordance with the population catered. [6,30-33] Patients with microcytic hypochromic anaemia could be easily screened out for β – thalassaemia minor and iron deficiency anaemia through these discrimination indices in the absence of other complicated diseases. [34] Useful indicator of β – thalassaemia minor identified was MCV and MCH less than 70 fl and 25 pg respectively, with normal or slightly decreased haemoglobin and raised red cell count greater than 5.0 X 10^{12} / l. [35] The goal of a reliable screening test is to get as close as possible to zero false negative result with a minimal percentage of false positive results. Too many false negative can make a screening parameter unreliable . A big confounding variable is β – thalassaemia carriers with a normal or near normal blood count indices. [3] In β – thalassaemia minor cases reduction in MCV and MCH did not correlate with the degree of anaemia , while red blood cells count is usually more than 5X 10^{9} / l. [34,36] Mean MCV, MCH and RBC values in present study are closely related with study of Yousafzai YM (2010). [1] A high MCV is known to be characteristic of specific mutations with milder disease . These cases with a MCV >75 fl are likely to give a false negative result on different discriminating variable. [30]. MCH is found as a more significant distinguishing feature among thalassaemics. [39,40] The application of Youden index can further strengthen the specificity and sensitivity of a discriminating factor .[8] An appropriate discriminating factor is the one which separates individuals with β – thalassaemia minor from those without β – thalassaemia minor , regardless of their iron status. [37] Results of present study are substantiated by Porprasert S et al (2014), who showed that Srivastava and Sirdah formulae have 100% sensitivity and negative predictive value, the highest

efficiency (97.4%0 and the highest Youden's index value (96.4%). [38]

Conclusion

Careful analysis of red blood cells indices, along with applying discriminating factors , can help to make a substantial guess about β –thalassaemia minor .

References

1. Yousafzai YM, Khan S, Raziq F. β –thalassaemia trait – Haematological parameters . J Ayub Med Coll Abbotabad 2010; 22(4): 84-86
2. Khatak SA, Ahmed S, Jalal A, Hafeez R. Prevalence of beta thalassaemia trait in Pakistan. JPMA 2012;62(1: 40-43
3. Roth IL, LAchover B, Koren G, Levin C. Detection of β –thalassaemia carriers by red cell parameters obtained from automatic counters using mathematical formulas . Mediterr J Hematol Infect Dis 2018; 10(1): e2018008
4. Sirdah M, Tarazi I, Najjar EA,Alhaddad R. Evaluation of diagnostic reliability of different RBC indices and formulas in the differentiation of β –thalassaemia minor from iron deficiency. Int J of Lab Haematology 2008; 30(40: 324-30
5. Sirachianan N, Iamsirirak P, Charoenkwan P, kadegasem P, Koon P W. New mathematical formulas for differentiating thalassaemia trait and iron deficiency anaemia in thalassaemia prevalent area. Southeast Asian J Trop Med Public Health 2014; 45(1): 174-82
6. Miri-Moghaddam E, Sargolzale W. Cut off determination indices in differential diagnosis between iron deficiency anemia and β –thalassaemia minor. International Journal of Hematology Oncology 2014;892)27-32
7. Sahli CA, Bibi A, Ouali F, Fredj SH. Red cell indices differentiation between beta thalassaemia trait and iron deficiency . Clin Chem Lab Med 2015; 51(11): 2115-24
8. Vehapolu A, ozgurhan G, Demir AD. Haematological indices for differential diagnosis of beta thalassaemia trait and iron deficiency . Anemia 2014; 2014: 576738
9. 9.Sargotzaie N, Miri-Moghaddan E. A local equation for differential diagnosis of beta thalassaemia trait and iron deficiency anaemias by logistic regression in Southeast Iran. Hemoglobin 2014; 38(5): 355-58
10. Urrechaga E, Agurre U, Izquierdo S. Mutivariate discriminant analysis for the differential diagnossi of microcytic anaemias. Anemia 2013; 2013: 457834
11. Zaghlone A, Al-Bukhari TA, Bajnaifer N, Al-Pakistani HA. Introduction of new formulas and evaluation of the previous red blood cell indices and formulas in the differentiation between beta thalassaemia trait and iron deficiency anaemia in the Makkah region. Hematology 2016; 21(6): 351-58
12. Bordban E, Tagliporn M, Zucconi BE. Reliability of different RBC indices and formulas in discriminating between thalassaemia minor and other microcytic hypochromic cases. Mediterr J Hematol Infect Dis 2015; 7(1): e2015022
13. Hisham A, Gettal HAY, Hameed M. Said Hi and He are new indices in differentiation between iron deficiency anaemia and beta thalassaemia trait. Iran J of Dentl and Med Sci 2015;14(7):67-72
14. Hoffman JJ, Urrechage E, Agnirre U. Discriminant indices for distinguishing thalassaemia and iron deficiency in patients with microcytic anaemia : a meta analysis. Clin Chem Lab Med 2015; 53(12): 1883- 94
15. Schoorl M, vanPelt J, Bartles PC. Application of innovative hemocytometric parameters and algorithms for improvement of microcytic anaemia discrimination. Hematol Rep 2015; 7(2): 5843
16. Srivastana PC. Differentiation of thalassaemia minor from iron deficiency . Lancet 1973; 2(7821): 154-55
17. Nesa A, Munir SF, Sultana T, Rahman MQ. Role of discrimination indices in differentiation of beta thalassaemia trait and iron deficiency anaemia . Mymensingh Med J. 2011;20(1): 1110-04
18. Mentzer WC. Differentiation of iron deficiency for thalassaemia trait. Lancet 1973; 1(7808): 882-85
19. Ehsani MA, Shahgholi MS, Rahiminejaid F. A new index for discrimination between iron deficiency and beta thalassaemia minor. Pak J of Biol Sci 2009; 12(5): 473-75
20. Ismail M, Nisar PG. Evaluation of the diagnostic accuracy of twelve discrimination indices for differentiating β –thalassaemia trait from iron deficiency anaemia. Indian J of Public Health, Research and Development 2106; 7(1): 104-07
21. Cao A, Rosatelli MC, Monni G, Galanello R. Screening for thalassaemia : a model of success . Obstet Gynecol Clin North Am 2002;20(2): 325-28
22. Shine I, lal S. A strategy to detect β –thalassaemia minor . Lancet 1977; 1(8013): 692-94
23. England JM, Brai BJ, Fraser PM. Differentiation of iron deficiency from thalassaemia trait . Lancet 1973; 1(7818): 1514-17
24. Green R, King R. A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anaemia from thalassemia minor. Blood Cells 1989; 3(15): 481–95
25. Novak RW. Red cell distribution width in pediatric microcytic anaemias . Pediatrics 1987; 80(2): 251-54
26. Ricerca BM, Storti S, d'Onofrio G. Differentiation of iron deficiency from thalassaemia trait : a new approach . Haematoogica 1987; 72(5): 409-13
27. Keikhaei B. A new valid formula in differentiating iron deficiency anaemia from β –thalassaemia trait. Pak J med Sci 2012; 26(2) 368-73
28. Telmissani OA, Khalil S, George TF. Mean density of hemoglobin per liter of blood: a new haematoligic parameter with an inherent discriminant function. Laboratory Haematology 1999; 5: 149-52
29. Koren A,Profeta L, Zalman L, palmor H. Prevention of beta thalassaemia in Northern Israel-a cost benefit analysis. Mediterr J hematol Infect Dis 2014; 6(1): e2014102
30. Moghaddani EM, Naderi M, Izadi S, Mashhadi M. Causes of new cases of major thalassaemia in detection of beta thalassaemia trait in areas of high prevalence . Iran J Public Health. 2012; 41(11): 67–71
31. Rund D, Filon D, Strauss N. Mean corpuscular volume of heterozygotes for beta thalassaemia correlates with the severity of mutations. Blood 1992; 79(1) : 238-43
32. Ntaios G, Chatzinikolaou A, Saouli Z. Discrimination indices as screening tests for beta thalassaemia trait. Ann Hematol 2007; 86(7): 487-91
33. Matos JF, Dusse LM, Stunnert RV. Comparison of discriminative indices from iron deficiency anaemia and beta thalassaemia trait in a Brazilian population . hematology 2013; 18(30: 169-74
34. Shen C, Jiang YM, Shi H. Evaluation of indices in differentiation between iron deficiency anaemia and beta thalassaemia trait for Chinese children. J Paediatr Hematol Oncol 2010; 32(6): 218-22
35. Nesa A, Munir SF, Sultana T. Role of discrimination indices in differentiation of beta thalassaemia trait and iron deficiency anaemia . Mymensingu Med J 2011;20(1): 110-14
36. Meraj F, Jamal S. Vlae of red cell parameters and peripheral smear in diagnosis of β –thalassaemia trait. Haemcon 2009;Karachi:12
37. Rathod DA, Kaur A, Patel V. Usefulness of cell counter based parameters and formulas in detection of beta thalassaemia trait in areas of high prevalence . Am J Clin Pathol 2007; 585-89
38. .Amid A, haghi –Ashtiani H, Allen K. Screening for thalassaemia carriers in populations with a high rate of iron deficiency: Revisiting the applicability of Mentzer Index and the effect of iron deficiency in HbA2 levels. Hemoglobin 2015; 39(2):141-43
39. Porprasert S, Panya A, Punyammugh M, Yanole J. Red cell indices and formulas used in differentiation of β –thalassaemia trait. Am J Clin Pathol 2012; 13892): 300-04
40. Mehdi SR, Al Dahmash BA. A comparative study of hematological parameters of β and α thalassaemia in a high prevalence zone: Saudi Arabia. Indian J Hum Genet 2011; 17(3): 207-11