Value Of Discrimination Indices In Screening Of Beta Thalassaemia Trait

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Abstract

Beta-thalassaemia trait (BTT) is one of the common causes of microcytic hypochromic anaemia. Several discrimination indices have been introduced to screen out BTT and discriminate them from other microcytic hypochromic anaemia. These discrimination indices were calculated from several cell counter bases parameters provided by automated haematology analyzer. The purpose of the study was to evaluate the value of five discrimination indices in screening of BTT. This study consists of 57 cases (HbA2 >3.5%) of BTT. Five discrimination indices evaluated in this study for detection of BTT were RBC count, red blood cell distribution width index (RDWI), Green & King index (G & K), Mentzer index (MI) and England & Fraser (E & F) index. Sensitivity, specificity, positive and negative predictive values and Youden's index had been calculated. RBC count and RDWI appears to be reliable and useful index for the detection of BTT as both of them had >80% sensitivity and specificity in detection of BTT. Other indices had high specificity but low sensitivity for detection of BTT, which were not suitable for screening test. From this study it can be concluded that, patients with microcytic hypochromic anaemia could be easily screened out for BTT through RBC count and RDWI, in the absence of other complicated diseases.

Key words: *Beta thalassaemia, discrimination indices*

Introduction

Thalassaemia is the most prevalent inherited disorder of Hb synthesis and it is a problem in many areas of the world, including south East Asia[1]. The term thalassaemia refers to a group of blood diseases characterized by decreased synthesis of one of the two types of polypeptide chains (α or β) in the haemoglobin molecule, which form the normal adult human haemoglobin molecule (HbA-$\alpha_2\beta_2$)[2-3]. Depending on the involved genes, the defect is identified as α thalassaemia or β thalassaemia. It is estimated that 1.5% of the world population carries β thalassaemia, i.e, at least 80 to 90 million people with an estimated 60,000 new carriers born each year. In which the south East Asia region accounts for about 50% of the world carriers[4].

Bangladesh also lies in thalassaemia belt and it is one of the most common inherited diseases in Bangladesh[5]. WHO estimates that 3% of our populations are carriers of beta thalassaemia, i.e, 3-6 million beta thalassaemia trait (BTT) in Bangladesh and affected births per thousand of BTT are 0.106, i.e, more than two thousand thalassaemic children are born every year in Bangladesh[3]. Beta thalassaemia in the homozygous condition causes profound anaemia that kills untreated affected children before 2 years, however patient treated with regular blood transfusion or bone marrow transplantation is approaching near normal life expectancy. WHO bulletin, 2008 showed; annually about 56,000 births had a major thalassaemia including at least 30,000 who need regular transfusions to survive. About 100,000 patients are currently living with regular transfusions and at least 3,000 die annually in their teens or 20s from uncontrolled iron overload[6]. So it is necessary to screen out beta thalassaemia trait to prevent homozygous birth. One way of achieving this goal is to screen the population at risk and instruct the identified heterozygous carriers about the genetic implications of marrying another carrier[7].

Most of the BTT is asymptomatic and they may not be aware of their carrier state. BTT is associated with mild or no anaemia but with reduced mean

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corpuscular volume (MCV), mean cell haemoglobin (MCH) and raised level of HbA_2[4,6].

Estimation of Hb A2 by high performance liquid chromatography (HPLC) is considered as standard method of diagnosis of BTT, but it is costly and not available routinely[4]. The accepted protocol for diagnosis of thalassaemia minor in routine haematology laboratories is performance of Hb electrophoresis, HbA2 quantification[8] HbA2 3.5% is considered as beta thalassaemia trait[9] However, in some mutations of BTT, HbA_2 is not elevated and it is a costly and time-consuming test[10]. With the advent of electronic cell counter, estimation of red cell indices become attractive and several discrimination indices can be used to screen out BTT. It is rapid, automated and less expensive method[11]. These calculations are based on the fact that microcytosis is usually more prominent than anaemia in thalassaemia trait and these are useful in the evaluation of uncomplicated cases[12].

Materials and Methods

This present cross-sectional study was carried out in the Department of Clinical Pathology, BSMMU-Dhaka in collaboration with Department of Haemato-onchology and Haematology, BSMMU- Dhaka. The newly clinically diagnosed cases of BTT were selected according to inclusion criteria. A total 57 cases were selected as beta thalassaemia trait on the basis of Hb A2 estimation by Hb electrophoresis. Patients with HbA2 more than 3.5% were identified as BTT cases (Figure-II shows the electrophoretic patteren of HbA2 in case of BTT) . Then complete blood counts including red cell indices were obtained by automated haematology analyzer in all the patients. Following cell counter based formulas were applied in all cases:

RBC count (10^{12}/L)

Mentzer Index (MI) : MCV/RBC[13].

England and Fraser Index (E&F): MCV - RBC - (5 xHb) - k[14]. [In the counter used in this study k was calculated to be 8.4]

Green and King Index (G & K) : MCV^2 RDW/100 xHb[15].

RDW Index (RDWI): MCV RDW xRBC[16].

The value of different discrimination indices considered as BTT were given in table-I. Then all the data were analyzed by standard statistical methods using SPSS 16 software. Validity of different discrimination indices in detection of BTT were evaluated by calculating their sensitivity, specificity and Youden's index. The sensitivity and specificity, PPV, NPV and Youden's index were calculated as follows:

Sensitivity = True positive/ (true positive + false negative)

Specificity =True negative/ (true negative + false positive)

PPV =True positive/(true positive + false positive)

NPV=True negative=(true negative + false negative)

Youden's index = sensitivity +specificity -100.

Table-I

Discrimination indices used in evaluation of BTT

Indices	In favor of BTT
RBC	>5
MI	<13
E & F	negative
G & K	<73
RDWI	<220

Results

The mean haematological data in BTT cases were plotted in table-II. In the current study, RBC count showed the high sensitivity and specificity for detection of BTT, it was 82.46% and 88.89%, which were statistically significant (P value is <0.001).

RDWI also prove its potentiality as a screening test, as it had sensitivity, specificity, and YI, 80.7%, 84.72% and 65.42 % respectively for detection of BTT cases. Other indices showed high specificity but low sensitivity for detection of BTT, which were not suitable as screening test. Youdens index (YI) gives an appropriate measure of validity of a particular technique. YI of RBC count was found the highest with the value of 71.35, which could be most reliable discrimination index for detection of β thalassaemia trait. From the current study Youden's index of five discrimination indices, from highest to lowest values were as follows: RBC count > RDWI > MI > E & F > G & K. Table III shows the sensitivity, specificity, positive predictive value, negative predictive value and Youdens index of different discrimination index for detection of BTT.

Table-II

Statistics of different values of blood indices in BTT cases

Group	N	Test	Mean	Std Devi-ation	Significance (P Value)
BTT	57	Hb (gm/dl)	10.17	±0.84	<0.001
		MCV (fl)	63.90	±7.49	(highly
		MCH (pg)	19.92	±2.88	significant)
		MCHC (gm/dl)	31.06	±1.61	
		RDW (%)	16.41	±2.46	

Table-III

discrimination indices in diagnosis of BTT

Indices	Differential value	Cases (%)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden's index
RBC Count	>5	BTT	82.46	88.89	85.45	86.49	71.35
RDWI(MCV xRDW/ RBC)	<220	BTT	80.70	84.72	80.70	81.33	65.42
MI (MCV/ RBC)	<13	BTT	71.93	88.89	83.76	80.00	60.82
G&K(MCV^2x RDW/100 x Hb)	<73	BTT	73.69	81.94	76.36	79.73	55.63
E&F[MCV RBC- (5 x Hb)-K]	<0 (- ve)	BTT	61.40	94.44	89.74	75.56	55.84

Discussion

Thalassaemia is one of the most common hereditary diseases. One-way of prevention of this disease is carrier detection and awareness among the disease[3]. The majority of BTT individuals are asymptomatic and unless diagnosed may be unaware of their carrier state and sometimes misdiagnosed as IDA. Morphologically on peripheral blood film it mimics so closely with IDA that sometimes it becomes difficult to differentiate them. Figure-1 shows features of peripheral blood film of BTT patients. Detection of BTT is important because MCV will not normalize in BTT if misdiagnosed as IDA and treated with iron[4]. In the present study it was found that, the BTT group had significantly lower values of MCV and MCH ($p<0.001$). Several studies have derived discriminant functions (such as, RBC count, E & F index, G & K index, RDW index, Mentzer index) based on RBC indices, can be used to distinguish these two condition in a cheaper and easier way[13-14].

Some authors reported the sensitivity of these indices were up to 100% in detection of BTT .13-16 But later on other studies estimated these indices sensitivity were between 61 to 91%[10]. There are remarkable inconsistencies among the results obtained in different studies. In the present study, the validity of discrimination indices in detection of BTT were evaluated by calculating their sensitivity, specificity and Youden's index. In this study, RBC count and RDWI came out as good index in detection of BTT, had both sensitivity and specificity more than 80%. This result is consistent with the findings of Demir et al. (2002). E & F index was found very high (94%) specificity for detection of BTT but sensitivity was poor, only 61%. Similar trend of findings also reported by Ntaios et al. (2007). Youdens index (YI) takes into account both sensitivity and specificity and gives an appropriate measures of validity of a particular technique. YI of RBC count was found highest among the five evaluated indices with the value of 71.35, which could be most reliable index for screening of ? thalassaemia trait. Beyan et al. (2007) also reported similar trend of findings. From this study it can be concluded that, RBC count and RDWI appears to be reliable and useful index for screening of BTT. So patients with microcytic hypochromic anaemia could be easily screened out for BTT through these discrimination indices in the absence of other complicated diseases. Despite the fact that, these indices cannot be conclusive for diagnosis of Beta thalassamia trait. For final diagnosis of Beta thalassamia trait Hb A2 estimation should be done.

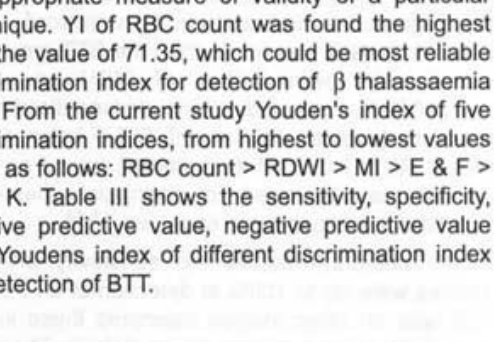

Figure I: PBF of case of BTT showing many target cells (X 400).

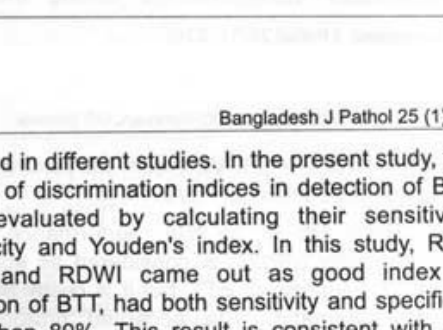

Figure II: Hb electrophoretis of case of BTT, showing raised Hb A2.

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References

1. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratana T, Changtrakul Y, Ukosanakarn, et al. A Reliable Screening Protocol for Thalassaemia and Hemoglobinopathies. Am J Clin Pathol 2005;123(1) :113-18.
2. Jimenez CV. Iron-Deficiency Anaemia and ThalassaemiaTrait Differentiated by Simple Hematological Tests and Serum Iron Concentrations. CLIN CHEM 1993;39(11):2271-75.
3. Ahmed J, Seraj UM, Chowdhury MA and Choudhury S. An Epidemiological Study of Thalassaemia, its Quick Diagnosis and Influence of Malaria in Chittagong Area of Bangladesh. Pakistan Journal of Biological Sciences 2004; 7 (11):1953-7.
4. Rathod DA, Kaur A, Patel V, Patel K, Kabrawala R, Patel V. Usefulness of cell-counter based parameters and formulas in detection of -thalassaemia trait in areas of high prevalence. AM J Clin Pathol 2007; 128:585-9.
5. Haque MS, Alam MA, Khan WA. Thalassaemia - Situation in Dhaka Shishu Hospital. Dhaka Shishu Hospital Journal 1999;15:30-36.
6. Modell B and Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bulletin of World Health Organization. 2008;86(6):480-87.
7. Shalev O, Yehezkel E and Rachmilewitz EA. Inadequate Utilization of Routine Electronic RBC Counts to Identify Beta Thalassaemia Carriers. American Journal of Public Health 1981;78(11):1476-77.
8. Lafferty JD, Crowther MA, Ali MA and Levine M. The Evaluation of Various Mathematical RBC Indices and Their E?cacy in Discriminating Between Thalassemic and Non-Thalassemic Microcytosis. Am J Clin Pathol 1996;106:201-5.
9. Alsaeed AH. Evaluation of Two Hematologic Indices and Extrapolated HbA2 Values in the Differential Dignosis of Iron Deficiency Anaemia (IDA) and Beta Thalassaemia Traits with IDA. International Journal of Hematology and Oncology 2008;18(4):226-33.
10. Demir A, Yarali N, Fisgin T, Duru F, Kara A. Most reliable indices in differentiation between thalassaemia trait and iron deficiency anaemia. Pediatr Int. 2002;44:612-6.
11. Shine I and Lal S. A strategy to detect beta-thalassaemia minor. Lancet. 1977;1:692-4.
12. Johnson CS, Tegos C and Beutler E. Thalassaemia Minor: Routine Erythrocyte Measurements and Differentiation from Iron Deficiency. Am J Clin Pathol 1983;80(1):31-36.
13. Mentzer WC. Differentiation of iron deficiency from thalas saemia trait. Lancet 1973;1: 882.
14. England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood-count. Lancet 1973; 1 : 449-52.
15. Green R, King R. A new red blood cell discriminant incor- porating volume dispersion for differentiating iron deficiency anaemia from thalassaemia minor. Blood Cells 1989; 15: 481-95.
16. Jayabose S, Giavanelli J, Levendoglu-Tugal O, Sandoval C, Özkaynak F, Visintainer P. Differentiating iron deficiency index. J. Pediatr. Hematol 1999; 21 : 314.
17. Olivieri NF. The beta-thalassaemias. N. Engl. J. Med 1999; 341 : 99-109