The *Thal-index* with the *BTT prediction.exe* to discriminate β-thalassaemia traits from other microcytic anaemias

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Abstract

Several attempts have been made previously to differentiate β-thalassaemia trait (BTT) from other microcytic anaemias using formulae with red cell (RC) parameters. Presently available formulae have low sensitivity and specificity. We wanted to develop a more precise algorithm, which could be used in situations where the gold-standard test for thalassaemia diagnosis: the high performance liquid chromatography (HPLC) is not available. The study was carried out prospectively from November 2008 to March 2010 from randomly collected blood samples with a mean cell volume (MCV) of less than 80 fL. HbA2 measured by HPLC was used to diagnose BTT. We used Fishers stepwise linear discriminant function analysis to develop an algorithm with RC parameters. Calculated new index *Thal-index* was then subjected to receiver operating characteristic curve analysis to identify best cutoff to discriminate BTT from other microcytic blood films. Software was developed to predict the BTT status (BTT prediction.exe). New index, referred to as the *Thal-index*, was calculated using discriminant function analysis and is given as *Thal-index*=[(0.615×MCV) +(0.518×mean corpuscular hemoglobin)+ (0.446×red cell distribution width)]. A value of 59 for *Thal-index* has 90% sensitivity and 85% specificity for differentiating BTT from other microcytic anaemias. This showed better sensitivity and specificity compared to other formulae presently used (*i.e.*, Mentzer in Eshani, *et al.*). Our study gives a better answer to set-up where HPLC is not available. Although this cannot replace HPLC, BTT prediction.exe is useful to predict instantly and is the first ever computer program available for this function.

Introduction

β-thalassemia trait (BTT) is one of the common causes of hypo-chromic microcytic anemias and differentiating it from the more common iron deficiency anaemia and other causes such as alpaha thalassaemia and sideroblastic anaemia without using sophisticated equipment has always been a challenge in low resource settings.

Several formulae using red cell characteristics are already available which attempts to differentiate these anaemias with varying successes.[1-4] We wanted to improve the rate of differentiation by using discriminant function analysis which is a technique used to build a predictive model of group membership based on observed characteristics of each case.[5]

A technique based on discriminant function analysis is not currently in use, nor is there easy to use computer software, which helps to differentiate BTT from other types of hypochromic microcytic anaemias.

We developed a new discriminant function using the red blood cell (RBC) parameters, which we would like to introduce as *Thal-index* as well as a computer program (BTT prediction.exe).

Materials and Methods

The study was carried out prospectively from November 2008 to March 2010 at Thalassaemia Unit at Ragama, Sri Lanka. All blood samples, which were referred for evaluation to the Thalassaemia Unit with a mean cell volume (MCV) less than 80 fL irrespective of haemoglobin level, were included consecutively to the sampling frame and 326 were selected randomly for the analysis.

Complete blood counts were performed using a *Beckman coulter A^{C}.T diff.2™* analyzer 2002 (Block Scientific Incorporation, Bohemia, New York, USA) in all 326. Blood levels of HbA2 were determined using Chromsystems by high-performance liquid chromatography variant T™ analyzer 2000 (Bio-Rad Laboratory, Hercules, California, USA) in all 326. HbA2 levels equal to or more than 3.5 was considered as BTT but the higher values were compared with other parameters such as HbF and other haemoglobinopathies were excluded from the analysis. RBC indices were compared irrespective of HbA2 levels and significances were calculated between the BTT and other microcytic blood films [RBC count, hemoglobin level, haematocrit, MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration, and red cell distribution width (RDW)]. Stepwise multivariate discriminant analysis determined those indices that best differentiated the above 2 groups after HbA2 assay. The new index was calculated and its sensitivity and specificity was compared with the following 4 indices: England-Fraser=MCV-RBC-(5×Hb)-k, Mentzer=MCV/RBC, Srivastava=MCH/RBC, Green and King (G&K) index=MCV2×RDW/ 100Hb.[3] Sensitivity and specificity were calculated.

The new index cut off point was determined by plotting a receiver operating characteristics curve and the best values were taken into consideration and a computer program was developed to get the thalassaemia status directly from the three RBC indices.

Results

There were 326 samples of which 232 were diagnosed as having β-thalassaemia trait and the remainder as other anaemias. BTT group had mean HbA2 levels 4.7 (SD 0.54), other cohort had 2.53 (SD 0.32). Table 1 illustrates the significances of RBC parameters between the two cohorts.

Discriminant analysis identified MCV, MCH and RDW as the best set of indices for differentiating the 2 diagnoses. The new index, referred to as the *Thal-index*, was calculated using discriminant function analysis and formula is given as:

Thal-index=(0.615×MCV)+(0.518×MCH)+ (0.446 x RDW).

The *Thal-index* gave 59.0 as the cutoff where 59 or below given as BTT. It has a sensitivity of 90% and a specificity of 85%.

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Discussion

Most of the RBC parameters did show a statistically significant difference between the two microcytic groups, which has been showed by previous similar studies (Table 1). Nevertheless, only MCV, MCH and RDW were taken into the stepwise linear discriminant function analysis although the RDW was not statistically significant. Almost all the indices had better sensitivities but the specificity was not as good as the new *Thal-index* (Table 2).

The new index we calculated developed with the discriminant function analysis gave a better Youden's index (75%). It was second to the Mentzer index performed by Ehsani, *et al.* when considering sensitivity alone and it is the index that gives the best specificity of the similar studies. Using our new formula is a reliable alternative to high performance liquid chromatography in situations where resources are limited, until the technology is established.

Table 1. The significance of red blood cell parameters between β-thalassaemia trait and other microcytic anaemias.

RBC parameter	Thalassaemia trait		Other anaemias		Significance
	Mean	SD	Mean	SD	
RBC (×10⁶/mL)	5.3922	0.8073	4.9870	0.6685	0.0001
HB (g/dL)	10.5208	1.4101	11.4769	3.3224	0.003
HCT (%)	33.7624	4.4928	34.9593	3.7663	0.12
MCV (fL)	62.7310	5.5030	70.7736	4.0353	0.0001
MCH (pg/cell)	19.6841	1.8734	22.5824	1.8662	0.0001
MCHC (g/dL)	31.1296	1.1264	31.8341	1.2833	0.0001
RDW (%)	15.9181	2.7778	16.1802	4.6532	0.413

RBC, red blood cell; HB, haemoglobin; HCT, haematocrit; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width.

Table 2. Comparison of different indices used in the study.

Parameters	Sensitivity %	Specificity %
Ehsani, *et al.*[4]	92.3	56.7
England-Fraser	77.0	42.9
Mentzer	90.3	58.1
Srivastava	87.8	57.5
Green and King	80.4	49.5
Thal-index[*]	90	85

[*]Index developed by authors of the current study.

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