Red cell parameters, HbA₂ levels, and the best discrimination index in beta thalassaemia trait in Sri Lanka

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Abstract

Introduction: Beta thalassaemia trait (BTT), also known as thalassaemia carrier state, leads to hypochromic microcytic indices. Identifying asymptomatic carriers on full blood count will help medical personnel to direct them for confirmatory tests such as High Performance Liquid Chromatography (HPLC) and prevent births of babies with thalassaemia major by discouraging intermarriage between two individuals with BTT.

Objectives: To describe red cell parameters and HbA₂ levels of individuals with BTT in Sri Lanka and determine the best discrimination index for screening of BTT.

Methods: BTT confirmed by HPLC (HbA₂ ≥3.5%) during a two year period at the Medical Research Institute, Colombo, Sri Lanka were selected and full blood count (FBC) reports were analysed. Haemoglobin (Hb), RBC parameters; RBC count, MCV, MCH, MCHC, and RDW and HBA₂ of the study population was analysed and the mean and the standard deviation (SD) was calculated for each parameter. These parameters were then analysed using Mentzer, Srivastava, England and Fraser, Green and King and Thal Index and sensitivity of each index for detecting BTT was calculated to ascertain which index identified BTT with maximum sensitivity.

Results: 157 BTT cases without concurrent iron deficiency and 206 BTT without serum ferritin results were analysed. In the BTT group without iron deficiency, Hb was 8.3-11.1 g/dl; mean 9.7 g/dl, RBC count 4.1-5.7; mean 4.9. MCV 59.2-68.8 fl; mean 64 fl. MCH 18.1-21.9 pg; mean 20 pg. MCHC 29.4-32.6 g/dl; mean 31 g/dl. RDW 12.2-23% mean 17.6%. HBA₂ level for BTT in Sri Lanka was 4.8% (SD 0.5).

Sensitivity for each index was, Mentzer Index 57.1%; England and Fraser Index 25.3%; Srivastava Index 44.2%; Green and King Index 61.5% Thal Index 73.3%; Shine and Lal Index 98.7% and New index was 92.3%.

Conclusions: All RBC parameters except MCHC were outside the normal ranges. A mean MCV of 64 fl (SD 4.8) and MCH of 20 (SD 1.9) best denoted BTT on FBC. The lower limit of HbA₂ (4.3%) in BTT in Sri Lanka is slightly higher than the currently used diagnostic value of ≥ 3.5%. Shine and Lal Index with a sensitivity of 98.7% was the most reliable index to identify BTT in Sri Lanka. Vigilant application of above findings on FBC by first contact doctors will help to identify more thalassaemia carriers.

Key words: beta thalassaemia trait, screening, RBC parameters, full blood count, HbA₂ level Sri Lanka
Several discrimination indices have been derived to be used as formulae to help ascertain whether the RBC parameters suggest a thalassaemia carrier state. The objective of this study was to describe RBC parameters of BTT in Sri Lanka and to determine the best discrimination index that will help first contact doctors to suspect BTT and to direct them for confirmation. We also aimed to describe the range of HbA2 level for BTT in Sri Lanka.

Methods

This descriptive cross-sectional study was carried out at the MRI, Colombo, Sri Lanka. We retrieved FBC and HPLC reports of all individuals with BTT diagnosed by HPLC during years of 2014 and 2015 from the institutional data base of the MRI. Subjects with iron deficiency (serum ferritin level < 15 μg/l) and age less than 18 years were excluded. Individuals without serum ferritin but diagnosed as BTT were included in the study as a separate group. Sample characteristics of enrolled patients were analysed using descriptive statistical methods and presented as percentages or proportions depending on their scales of measurements. FBCs were analysed and haemoglobin (Hb), RBC count, MCV, MCH, MCHC, and red cell distribution width (RDW) were tabulated for BTT patients. The mean and ranges for Hb, RDW, MCV, MCH, MCHC and HbA2 were analysed. Furthermore, the efficacy of the discrimination indices was analysed by applying each index to the diagnosed patients and determining which index identified thalassaemia carriers with greater sensitivity. Discrimination indices that were evaluated in our study were, Mentzer’s Index (MCV/RBC), Srivastava Index (MCH/RBC), England and Frazer’s Index (MCV-RBC-(5Hb)-3.4), Shine and Lal Index (MCV2×MCH/100), Green and King Index (MCV×RDW/100Hb), Thal Index (0.615×MCV + (0.518×MCH) + (0.446 ×RDW))^20 and New Index which is a new slightly simple formula (80-MCV) × (27-MCH)^21

The protocol was approved by the Ethical Review Committee, Faculty of Medical Sciences, University of Sri Jayewardenepura and ethical committee of MRI.

Results

A total of three hundred sixty-three (363) BTT individuals confirmed by HPLC (HbA2 ≥ 3.5%) were enrolled. There were 236 (65.4%) women and majority (n=224, 61.7%) were between 18-33 years, while 114 (31.4%) were between 34-57 years and 25 (6.9%) were between 58-81 years. They were from 15 districts of Sri Lanka with the majority from Colombo (38%).
Depending on the availability of serum iron studies, the total BTTs were divided into two groups; 157 subjects with BTT without concurrent iron deficiency who had a serum ferritin level of >15 μg/l and 206 BTTs who did not have serum ferritin reports available.

The group with confirmed BTT with no concurrent iron deficiency (157) were analysed for the red cell parameters and HbA₂ levels. Since it could be argued that the other 206 may or may not have iron deficiency (because serum ferritin was not available) their data was not used to describe RBC parameters and HbA₂. However since this is the type of patient who will present to first contact doctors (BTT +/- iron deficiency), they were used as a separate group for application of discrimination indices.

Mean and ranges for RBC parameters for BTT in the study group

The mean, standard deviation (SD) and ranges of RBC parameters (RBC Count, Hb, MCV, MCH, MCHC, RDW) of BTT without concurrent iron deficiency was tabulated (Table 1).

### Table 1. Red cell parameters on FBC in patients with BTT without concurrent iron deficiency

| Parameter          | Mean | SD  | Range  |
|--------------------|------|-----|--------|
| Hb (g/dl)          | 9.7  | 1.4 | 8.3-11.1|
| RBC count (10¹²/l) | 4.9  | 0.8 | 4.1-5.7 |
| MCV (fl)           | 64   | 4.8 | 59.2-68.8|
| MCH (pg)           | 20   | 1.9 | 18.1-21.9|
| MCHC (g/dl)        | 31   | 1.6 | 29.4-32.6|
| RDW (%)            | 17.6 | 5.4 | 12.2-23  |

Range and mean of HbA₂ in BTT in the study population

The mean HbA₂ level for BTT of the study population was 4.8% (SD 0.5) with a range of 4.3-5.3%. Although the diagnostic value of HbA₂ for BTT is ≥3.5%, the lower limit of HbA₂ in the study population was slightly higher at 4.3%.

Comparison between FBC parameters of BTT and normal individuals

The FBC parameters of BTT individuals was compared with the normal ranges which are routinely used in at the MRI (Table 2).

### Table 2. Comparison between FBC parameters of BTT and normal individuals

| Parameter | Ranges for BTT | Normal range | t value |
|-----------|---------------|--------------|---------|
| Hb (g/dl) | 8.3-11.1      | 11.3-16.5    | -37.17  |
| RBC count (10¹²/l) | 4.1-5.7 | 3.6-5.6 | 4.88 |
| MCV (fl)  | 59.2-68.8     | 78-96        | -59.30  |
| MCH (pg)  | 18.1-21.9     | 27-34        | -67.79  |
| MCHC (g/dl) | 29.4-32.6  | 31-36        | -18.02  |
| RDW (%)   | 12.2-23       | 10-16.5      | 9.36    |

In BTT cases, all RBC parameters were outside the normal ranges and MCV and MCH were markedly reduced (Table 2). Hb was slightly lower than normal. RBC count and MCHC also overlapped with the normal ranges. RDW is comparatively slightly higher and also overlaps with normal values. MCV and MCH were the most affected parameters and the range in BTT was well separated from normal values with no overlap at all. Furthermore, they displayed very significant t values (t= -59.30, t= -67.79 respectively) indicating that mean MCV and MCH in BTT are significantly different to that of normal population.

Determining the best discriminating index for identifying BTT on a FBC

The sensitivity of each index in screening for BTT was calculated by applying the above formulae separately for all the RBC parameters in the two groups; 1) BTT without iron deficiency (normal serum ferritin) and 2) BTT where serum ferritin was not available (Table 3).

### Table 3. Red cell indices-based formulae to screen BTT

| Name of the formula | Formula | Cut off for Beta thalassaemia trait |
|---------------------|---------|-----------------------------------|
| Mentzer index       | MCV/RBC | <13                               |
| Srivastava          | MCH/RBC | <3.8                              |
| England and Fraser  | MCV(5Hb)-RBC-K (K=3.4) | <0     |
| Shine and Lal       | MCV²×MCH/100 | <1530 |
| Green and King      | MCV²×RDW/100Hb | <72    |
| New index           | (80-MCV×(24-MCH) | >44.76  |
| Thal index          | (0.615×MCV) + (0.518×MCH) + (0.446×RDW) | ≤59    |
As shown in table 4, in the group with BTT without iron deficiency the Shine and Lal index had the highest sensitivity (98.7%), followed by New index (92.3%) and Thal index (73.3%). Accordingly, sensitivity of seven discrimination indexes for Sri Lanka, from highest to lowest values were as follows. Shine & Lal > New > Thal > Green & King > Mentzer > Srivastava > England & Frazer.

Even among the BTT where serum ferritin was not available, the sensitivity of Shine and Lal index was the highest (99.5%). The other discrimination indices also showed the same order.

Based on these findings, Shine and Lal index was the formula with the best sensitivity for identifying BTT in Sri Lanka.

|                | BTT without concurrent IDA | BTT± IDA |
|----------------|----------------------------|----------|
|                | BTT (n=157)                | Percentage of correctly identified cases | Sensitivity of index | BTT (n=206) | Percentage of correctly identified cases | Sensitivity of index |
| Mentzer index  | Identified correctly as BTT| 88       | 57.1%     | 57.1%       | 149         | 73%       | 73%       |
|                | Incorrectly as Non BTT     | 66       |           |             | 55          |           |           |
| Srivastava Index| Identified correctly as BTT| 69       | 44.2%     | 44.2%       | 119         | 58%       | 58%       |
|                | Incorrectly as Non BTT     | 87       |           |             | 86          |           |           |
| England and Frazer index | Identified correctly as BTT | 39       | 25.3%     | 25.3%       | 92          | 45.1%     | 45.1%     |
|                | Incorrectly as Non BTT     | 115      |           |             | 112         |           |           |
| Shine and Lal index | Identified correctly as BTT | 153      | 98.7%     | 98.7%       | 202         | 99.1%     | 99.1%     |
|                | Incorrectly as Non BTT     | 02       |           |             | 01          |           |           |
| Green and King index | Identified correctly as BTT | 83       | 61.5%     | 61.5%       | 148         | 77.9%     | 77.9%     |
|                | Incorrectly as Non BTT     | 52       |           |             | 42          |           |           |
| Thal index     | Identified correctly as BTT| 99       | 73.3%     | 73.3%       | 147         | 82.6%     | 82.6%     |
|                | Incorrectly as Non BTT     | 36       |           |             | 31          |           |           |
| New index      | Identified correctly as BTT| 143      | 92.3%     | 92.3%       | 186         | 91.6%     | 91.6%     |
|                | Incorrectly as Non BTT     | 12       |           |             | 17          |           |           |
Discussion

This study included 363 individuals diagnosed as BTT at MRI during a two year period. In literature, studies to ascertain sensitivity of discrimination indices have been done on groups with a smaller sample size. For example, Mentzer index was tested on 103 cases and England and Frazer on 72 cases.

As mean values for RBC parameters may vary in different populations, we strived to describe the changes in RBC parameters for BTT in Sri Lanka. We used the group without concurrent iron deficiency as confirmed by iron studies to describe the ranges and mean values. In doing so we were able to give the mean values for individuals with the sole diagnosis of BTT.

The t values when calculated showed that all parameters were significantly different from normal values and MCV of 64 fl and MCH of 20 pg did not show any overlap with the normal range. Therefore, these parameters best denote BTT on FBC reports. Although HbA2 levels of ≥3.5% on HPLC is used as confirmatory of BTT, the mean HbA2 value for BTT in Sri Lanka was found to be 4.8% (SD 0.5). Our results match those of the only other Sri Lankan study where MCV, MCH, and HbA2 have similar observations. According to the results of this study MCV, MCH, and HbA2 were 62.7 fl (SD 5.5), 19.7 (SD 1.9), and 4.7 (SD 0.54) respectively.

Various formulae based on Hb, RBC Count, MCV, MCH, MCHC, RDW have been devised to differentiate BTT from other hypochromic microcytic anemias. We applied 7 formulae and estimated the sensitivity of each formula for screening of BTT. Accordingly, the Shine and Lal index with a sensitivity of 98.7% was found to be the most accurate. This was followed by new index (92.3%), Thal index (73.3%), Green and King Index (61.5%), Mentzer index (57.1%), Srivastava index (44.2%), England and Frazer index (25.3%) in decreasing order.

Since these discrimination indices were used on the BTT with no concurrent iron deficiency (n=157), we can safely conclude that using the Shine and Lal index will pick up 98.7% of BTT on FBC. The Thal index was derived by a Sri Lankan group of researchers and the sensitivity of this discriminating index was 73.3% when applied to our study population.

Other studies worldwide show similar results. For example, a study conducted using Indian population suggested 99.1% sensitivity for Shine and Lal index. Okan et al found that Shine and Lal index is the most sensitive index (91%) to identify BTT in Turkey.

A value of < 1530 when the Shine and Lal formula (MCV×MCH/100) is applied will help to identify possible BTT on FBC report. The new formulae [(80-MCV)×(27-MCH)] by a group of Iranian researchers showed a sensitivity of 92.3% in our
BTTs. The importance of this formula is the simplicity and user friendliness over Shine and Lal. As shown by our study, MCV and MCH were the most affected indices in BTT and the ranges of these parameters in BTT are at a different range to the normal ranges with no overlap. Therefore, formulae that use MCV and MCH (Shine and Lal index and new index) have good discriminative function compared with RBC count and RDW related formulae.

It is not known whether the group of 206 BTT without serum ferritin has concurrent iron deficiency or not. We also do not know the amount of contribution that iron deficiency will have on RBC indices. The best discriminating index for this group too was Shine and Lal with a sensitivity of 99.5%. This was followed by New index (91.6%) and Thal index (82.6%) similar to the group without concurrent iron deficiency. Therefore, we can assume that Shine and Lal index can be used even when the iron status of the individual is not known with a sensitivity of 99.5%.

Conclusion

The ranges for RBC parameters and HbA\textsubscript{2} levels for BTT in Sri Lanka have been described by this study. All RBC parameters except MCHC were outside the normal ranges, however a mean MCV of 64 fl (SD 4.5) and MCH of 19.9 (SD 0.2) best denote a BTT on FBC.

Shine and Lal Index is the most reliable index to identify BTT in Sri Lanka when iron deficiency has been ruled out. This is followed by the New index and Thal index. Based on the findings of our study, these formulae can be used with reasonable accuracy even in the absence of serum ferritin levels.

We conclude that RBC parameters on FBC and indices based formulae are reliable, rapid and cost effective measures that can be used effectively by first contact doctors to screen for BTT in Sri Lanka when confirmatory tests such as HPLC, Hb electrophoresis are not freely available and not affordable.

Limitations

With the availability of HPLC in certain hospitals, especially Kurunegala, the samples received at MRI were limited. Therefore the FBC reports do not adequately represent BTT island wide.

Reference ranges for normal population in Sri Lanka have not been determined and the indices we have described are the normal values used at MRI for the auto analyzer.

Declarations

Availability of data and materials: Patient de identified raw data are with the researchers can be made available.

Competing interests

No competing interest.

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Authors’ Contribution

KHBPF: conceptualization of the research, editing of the research proposal through all stages, writing up the manuscript, editing and formatting manuscript to journal specifications. HMJPH: Supervising data collection and analysis. LDU, PMNKS: Writing the project proposal, data collection and analysis, writing the project report. All authors have read and approved the final manuscript.

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