Clinica Chimica Acta 579 (2026) 120592

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

# Population-adjusted cut-off: A new approach for enhancing the diagnostic efficacy of hematological discrimination formulae for screening β-Thalassemia trait

Isuru Aravinda [a], Shashini Dharmasiri [a], Chathurika Sewwandi [a], Sinthu Karunaithas [b], Sonali Goonetilleke [c], Karunaithas Rasaratnam [a,*]

[a] Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Jaffna, Jaffna 40000, Sri Lanka
[b] Biosensors Laboratory, Department of Biomedical Engineering, Faculty of Engineering, Mahidol University, Phutthamonthon 4 Road, Salaya, Nakhon Pathom 73170, Thailand
[c] Regional Thalassemia Prevention and Treatment Centre, Teaching Hospital Anuradhapura, Anuradhapura 50000, Sri Lanka

ARTICLE INFO

*Keywords:*
β-thalassemia trait
Population-adjusted cut-off
Hematological discrimination formulae
Sri Lanka

ABSTRACT

Screening for β-thalassemia trait (βTT) is crucial for preventing β-thalassemia major in offspring. Although hematological discrimination formulae (HDF), developed using complete blood count parameters, are cost-effective tools for screening βTT, their performance varies across different populations. This study evaluated the performance of 32 HDF for screening βTT in the Sri Lankan population. Data were retrieved from laboratory databases and categorized into confirmed βTT and non-βTT groups based on high-performance liquid chromatography results. The βTT screening performance of the HDF was assessed using accuracy measurements, the receiver operating characteristic (ROC) curve, and Youden's index (YI). Furthermore, a population-adjusted cut-off was determined using the Index of Union (IU) method to optimize the predictive accuracy of HDF in screening βTT. Bordbar demonstrated excellent predictive performance in males (AUC = 0.908; YI = 0.815), while Shine & Lal, Kerman-I, Nishad, Sehgal, Bordbar, and Roth demonstrated high discriminative ability in females (AUC > 0.833; YI > 0.666). Applying a population-adjusted cut-off improved the βTT screening potential of Shine & Lal, Kerman-I, Nishad, Bordbar, and Roth in males (AUC > 0.911; YI > 0.822) and enhanced the performance of Kerman-II in females (AUC > 0.861; YI > 0.722). Notably, Shine & Lal (AUC = 0.937; YI > 0.873) and Nishad (AUC = 0.897; YI > 0.794) demonstrated the best performance for males and females, respectively, when a population-adjusted cut-off was applied for screening βTT. In conclusion, determining a population-adjusted cut-off is a new initiative to enhance the βTT screening performance of HDF across different populations.

## 1. Introduction

β-thalassemia is a heterogeneous group of genetic disorders causing a substantial number of morbidity and mortality, as well as a financial burden worldwide [1]. β-thalassemia, a genetic disorder characterized by a mutation in the β-globin gene, is classified into β-thalassemia major, β-thalassemia intermedia, and β-thalassemia trait (βTT) based on their clinical and laboratory findings [2,3]. Approximately 60,000 children are estimated to be born with β-thalassemia, with an additional 1.5 % of the global population being carriers annually [4,5]. β-thalassemia is the primary type of thalassemia in Sri Lanka, affecting 2.5 % of all three major ethnic groups [6]. Screening βTT and identifying its heterozygous variant hemoglobin (Hb) is crucial for preventing and controlling β-thalassemia major in offspring [7]. The βTT is suspected when observing the near-normal Hb level, slightly elevated red blood cell (RBC) count, and microcytic hypochromic cells in the evaluation of routine RBC indices and blood picture, but confirmation of βTT is performed by quantifying $HbA_2$ in high-performance liquid chromatography (HPLC) or capillary zone electrophoresis [8,9]. Besides, reverse transcriptase polymerase chain reaction (RT-PCR) or DNA sequencing is employed to detect specific mutations in βTT [10–12]. However, the availability and accessibility of these techniques are restricted to resource-limited countries due to high costs [13,14].

Over the past few decades, several hematological discrimination

* Corresponding author.
*E-mail address:* karunaithas@univ.jfn.ac.lk (K. Rasaratnam).

https://doi.org/10.1016/j.cca.2025.120592
Received 13 January 2025; Received in revised form 27 August 2025; Accepted 3 September 2025
Available online 5 September 2025