REVIEW ARTICLE

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Erythrocyte Indices for Beta-thalassemia Trait (BTT) Screening in Indonesia: A Scoping Review

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ABSTRACT

Background: Thalassemia is a monogenic disorder that ranks fifth among the catastrophic diseases in Indonesia. Thalassemia screening plays an important role in preventing the birth of individuals with thalassemia major. Numerous erythrocyte indices have been used as first-line screening methods for beta-thalassemia trait, preceding definitive analysis using hemoglobin electrophoresis.
Objective: This study aimed to conduct a literature review comparing the most frequently used erythrocyte indices in Indonesia with hemoglobin electrophoresis.
Methods: : A systematic search was conducted using PubMed, Scopus, EBSCO, and Google Scholar databases in July 2024. This study included full-text articles in Indonesian or English that compared erythrocyte indices and hemoglobin electrophoresis for thalassemia screening.
Results: The Six articles were included in this review. Two of the six articles analyzed the compatibility of erythrocyte indices and hemoglobin electrophoresis using kappa statistics. The remaining articles calculated the diagnostic values. Among the ten erythrocyte indices, the Mentzer index was the most frequently assessed, appearing in six studies, followed by the indices of Shine & Lal and Sirdah, each evaluated in three studies. The Green-King, England & Fraser, and Srivastava indices were each examined in two studies. Additionally, Ehsani, Matos and Carvalho, RDW, and MCV and/or MCH indices were each assessed in one study.
Conclusion: The compatibility between erythrocyte indices and hemoglobin electrophoresis, based on two studies, was fair indicating that hematological indices alone are insufficient for a definitive diagnosis. This finding aligns with the conclusions of four other studies, which also suggested that no single erythrocyte index is definitive. Among the indices, the Green-King Index demonstrated the highest reliability; however, further studies are needed to support this finding, while hemoglobin electrophoresis remains essential for an accurate diagnosis.

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1. Introduction

Thalassemia is the fifth most catastrophic disease in Indonesia that requires long-term and high-cost treatment.[1] This condition is a genetic disorder caused by reduced or loss of synthesis of alpha globin chains and/or beta globin chains. Beta-thalassemia is inherited in an autosomal recessive manner, resulting in carriers of the beta-thalassemia trait (carrier/minor). Beta-thalassemia carriers generally are asymptomatic. However, both couples with beta-thalassemia minor (carriers) have a 25% chance of having offspring with beta-thalassemia major, which requires regular transfusions. Therefore, thalassemia should be diagnosed as early as possible.[2,3]

Thalassemia screening is essential in preventing the emergence of new cases of thalassemia major and can be implemented during the premarital, preconception, or prenatal stages.[4] However, detecting thalassemia carriers in Indonesia, a lower-middle income country with a large population, vast geographical regions, and diverse cultural backgrounds, is challenging. Moreover, the prevalence of anemia caused by iron deficiency (iron deficiency anemia, IDA) is high in Indonesia, especially in children and young women.[5] Differentiating between IDA and thalassemia based solely on routine hematological blood counts is difficult, as both are associated with microcytic and hypochromic erythrocytes.[4]

Hematological parameters, followed by Hemoglobin A2 (HbA2) measurement, are vital for screening programs targeting beta-thalassemia. A High HbA2 level (≥3.5%) is generally used as the cut-off value for beta-thalassemia.[6,7] Nonetheless, DNA analysis remains the gold standard for identifying the specific type of mutation in patients with thalassemia. As a lower-middle-income country with a large population, HbA2 measurement as well as DNA analysis in

Indonesia is costly and not widely available in primary health care. Numerous erythrocyte indices have been introduced to help identify beta-thalassemia carriers because they are rapid to perform or cost-effective, although it is commonly agreed that none of the indices are 100% sensitive and specific.[4] To overcome this numerical limitation in Indonesia, we performed a scoping review to compare the performance of erythrocyte indices.

2. Methods

Literature Search

A systematic search was conducted in PubMed, Scopus, EBSCO, and Google Scholar using the combination of keywords with search functions and Boolean operator, i.e., thalassemia screening OR thalassemia screening program OR thalassemia prevention program OR thalassemia carrier screening AND thalassemia trait OR thalassemia carrier AND Indonesia to identify relevant articles published until July 2024. The formulation of the research question was based on the PICO method (Table 1).

Table 1. The PICO method

Population	Indonesian Population
Intervention	Erythrocyte indices examination
Control	HbA2 level examination
Outcome	Beta-Thalassemia carrier

A scoping review methodology was selected because it offers a systematic search process, predefined eligibility criteria, and a reproducible screening method, thereby enabling a clearer mapping of available evidence and identifying gaps in the literature. This approach supports a more reliable summary of existing findings while accommodating variations in study design and reporting.

Selection Criteria

All studies published in languages other than English or Bahasa Indonesia, as well as those on other hematological conditions or other diseases, laboratories, and geographic locations not in Indonesia, were removed before screening. After duplicate articles were removed, review articles, books, theses, grey literature, and abstract-only articles were excluded from this study. Only studies conducted on the Indonesian population were included in this review. Additional inclusion criteria were peer-reviewed original research articles that reported confirmatory erythrocyte indices along with HbA2 analysis for the screening of beta-thalassemia traits.

Data Collection

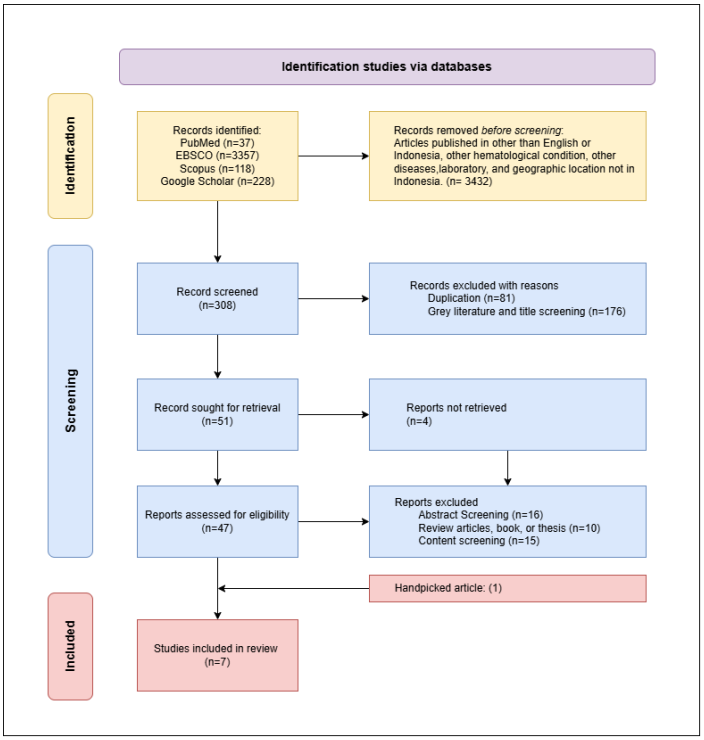
Data from various articles, including authors, titles, year of publication, number of samples, study location, erythrocyte indices, statistical analysis, and HbA2 levels, were collected using Microsoft Excel. The relevant data are written as (-) when they could not be obtained from the articles. Three reviewers independently performed screening followed by full-text assessment. Disagreements were resolved by consensus among the reviewers.

The articles were then screened for duplication and title screening was performed. Grey literature was excluded from the analysis as it did not constitute a journal and not meet the inclusion criteria. Fifty-one articles were retrieved, and 47 remained for full-text review. Further article exclusion was based on abstract screening (16), review articles, books, or theses (10), and content screening (15). We identified six articles in which at least one erythrocyte index was evaluated.

3. Result

The identification process of the studies screened and collected is shown in Figure 1. In total, 3,740 articles were obtained from the electronic databases. We removed 3,432 articles, including articles written in language other than English or Bahasa Indonesia, article on other hematological conditions or other diseases, and articles from laboratory and geographic locations not in Indonesia.

Figure 1. Flow diagram of identification, screening and inclusion articles



Two of the six studies analyzed the compatibility of erythrocyte indices and hemoglobin electrophoresis using the kappa statistic (Table 2).

Table 2. Compatibility test between erythrocyte indices and Hb-electrophoresis

Author	(1)	(2)
Sample Size	99	23
Mentzer	46.5%; K: 0.663	n=1; K: 0.228
England & Fraser	55.6%; K: 0.636	n=0; K: n.d
Shine & Lal	54.5%; K: 0.527	n=3; K: 0.251
Srivastatva	55.6%; K: 0.558	n=1; K:0.228
Sirdah	-	n=1; K:0.228
Ehsani	-	n=1; K:0.228
HbA2	47.5%	n=6

(1) Harahap RIM, et al; (2) Susanti, AI, et al; K: Kappa value; n.d: not determined; n: number of samples.

The two studies reported fair concordance between erythrocyte indices and hemoglobin electrophoresis for diagnosing beta-thalassemia traits.[8,9] Harahap, RIM, et al. found that 47.5% of their 99 participants were identified with BTT through hemoglobin electrophoresis. The Mentzer and England & Fraser indices demonstrated good alignment with the electrophoresis results, whereas the Shine & Lal and Srivastava indices showed moderate compatibility. Susanti et al. revealed that a complete blood count test was more reliable than a finger-prick test for detecting anemia in pregnant women. Although their study included 105 participants, only the 23 women who were anemic according to the CBC underwent HbA_2 testing, among whom six were identified as BTT. The variations in these results might be attributed to differences in study design, such as sample size and inclusion/exclusion criteria. The remaining four studies calculated various diagnostic performance metrics, including sensitivity, specificity, predictive value, and likelihood ratio (Table 3).[10–13]

The studies reviewed in Table 3 demonstrated the varying diagnostic performance of erythrocyte indices for screening beta-thalassemia carriers. The Green-King Index exhibited high sensitivity and specificity across the two investigations, with Indrasari et al. reporting 78.6% sensitivity and 76.6% specificity, while Salim et al. found even stronger values of 96.9% sensitivity and 67.5% specificity. In contrast, the Mentzer Index showed moderate accuracy, with Siswandari et al. documenting 81% specificity but a notably lower 36% sensitivity. The Shine & Lal Index, as reported by Sahiratmadja et al., had the strongest sensitivity at 96%, indicating a strong ability to detect thalassemia carriers, although its specificity was lower at 40.5%, suggesting a higher number of false positives.

Table 3. sensitivity, specificity, NPV, PPV, PLR, NLR, and accuracy

	1	2	3	4	5	6	7
Sensitivity							
a	91.8	83.6	96.9	92.8	-	-	-
b	-	66	78.6	64.2	27.7	-	-
c	-	36	-	-	-	-	-
d	-	83.80	-	-	-	96	38.6
Specificity							
a	75	66.2	67.5	58.7	-	-	-
b	-	62.5	76.7	64.1	25	-	-
c	-	81	-	-	-	-	-
d	-	82.2	-	-	-	40.5	67.6
NPV							
a	88.2	76.8	94.7	87	-	-	-
b	-	89.3	81,4	81,6	47.8	-	-
c	-	44	-	-	-	-	-
d	-	-	-	-	-	-	-
PPV							
a	81.8	75.2	78.5	73.3	-	-	-
b	-	59	42.6	41.8	12.2	-	-
c	-	75	-	-	-	-	-
d	-	-	-	-	-	-	-
PLR							
a	3.6	2.4	2.9	2.2			
b	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-
NLR							
a	0.1	0.2	0.04	0.1	-	-	-
b	-	-	-	-	-	-	-
c	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-
Accuracy							
a	-	-	-	-	-	-	-
b	-	78.03	65.02	64.13	26.9	-	-
c	-	-	-	-	-	-	-
d	-	-	-	-	-	-	-

a: Salim Y, et al; b: Indrasari, YN, et al; c: Siswandari W, et al; d: Sahiratmadja E, et al; 1: RDW; 2: Mentzer; 3: Green-King; 4: Sirdah; 5: Martos & Carvallo; 6: Shine & Lal; 7: MCV, MCH; NPV: Negative Predictive Value, PPV: Positive Predictive Value, PLR: Positive Likelihood Ration, NLR: Negative Likelihood Ratio, RDW: Red Cell Distribution Width, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin.

Differences in study design and methodology may have contributed to variations in diagnostic outcomes. Factors such as sample size, where Indrasari et al. (2021) studied 223 participants, while Siswandari et al. (2019) had a

smaller sample of 37 patients, may partially account for the disparities. Additionally, while all studies utilized hemoglobin analysis, Sahiratmadja et al. (2022) incorporated DNA sequencing, providing more robust confirmatory results than the reliance on hematological parameters alone.

4. Discussion

Preventive strategies for thalassemia are significantly more effective than treatment. One effective strategy involves screening and early detection within at-risk populations to avert marriages between individuals who are carriers of thalassemia, thereby reducing the likelihood of offspring with thalassemia major. Therefore, the early identification of thalassemia carriers is essential and is more appropriate to be implemented prospectively rather than retrospectively.[14]

The prospective screening approach entails comprehensive screening of the general population to identify carriers of thalassemia prior to and during the childbearing years, before the birth of an affected child. In contrast, targeted screening is focused on specific population groups, such as couples preparing for marriage, prior to conception, or during the early pregnancy period.[14] The number of new thalassemia major cases reduced to nearly zero annually in Crypus by implementing premarital screening, suggesting that the premarital stage is the most appropriate time to perform thalassemia screening.[4]

According to the Minister of Health of the Republic of Indonesia, the integration of screening and early detection for preschool children (2-6 years old) will be incorporated into the child health program at integrated health post (*pos pelayanan terpadu, posyandu*), with a particular focus on families with first-degree relatives affected by thalassemia major. This corresponds with the increased occurrence of BTT observed in families affected by thalassemia, as indicated by existing studies.[8,13] Alternatively, thalassemia screening can be performed in the neonatal period through a newborn screening program.[15] Summary of sample characterization of six studies are as follows (Table 4).

Table 4. Sample characterization of six studies

Author	Sample	Age Distribution	HbA2≥3.5%/ Hb Electrophoresis result
a	99; Transfusion-dependent thalassemia patient's family	0-14 y.o: 39 15-55 y.o: 57 >56 y.o: 3	Hb E-Trait: 18 (18%) Hb E: 6 (6%) BTT: 47 (47%)
b	23; Anemic pregnant women	Pregnant women	6 (26%)
c	178; IDA and BTT	Over 12 months	80 (62.5%)
d	223; IDA and BTT	3- 17 y.o	159 (71.3%)
e	37; Anemia patients	<17 y.o: 22 ≥17 y.o: 15	Hb E: 5 (13.5%) BTT: 6 (16.2%)
f	160; Sibling and extended family members of thalassemia major.	Unmarried group: 43.7% 5-11 y.o: 13.8% 12-15 y.o: 9.4% >15-24 y.o: 20.6% Married group: 56.3%	74 (46.25%)

a. Harahap RIM, et al; b. Susanti, AI, et al; c: Salim Y, et al; d: Indrasari, YN, et al; e: Siswandari W, et al; f: Sahiratmadja E, et al

These data presented the various age distributions and the presence of thalassemia traits (such as Hb E and BTT) across different groups, highlighting the importance of screening and early detection of thalassemia carriers especially in at-risk population as well as the importance of genetic counselling, given that thalassemia is an inherited disease.

These differences can influence apparent diagnostic outcomes through spectrum effects, in which indices may behave differently in populations dominated by IDA-related microcytosis versus those enriched for genetic hemoglobinopathies.[16] It also shows the overlap between IDA and BTT, which explicitly acknowledged as a major diagnostic challenge in the included studies. This may further shift test performance when the background prevalence and severity distribution of microcytosis differ between settings. The observed variability in sensitivity and specificity across indices (e.g., very high sensitivity but lower specificity for Shine & Lal in one cohort, versus mixed performance of Mentzer across studies) should therefore be interpreted as context-dependent, rather than as evidence that a single index universally outperforms others.

Beyond sample size differences, geographic and ethnic heterogeneity within Indonesia may also contribute to variation of study included, as regional differences in

hemoglobinopathy patterns (e.g., coexisting Hb E traits noted in certain cohorts) can alter red-cell parameter distributions and thereby affect index thresholds and misclassification rates.[17]

In regions where beta-thalassemia is prevalent, hemoglobin analysis is an essential diagnostic tool for carrier screening.[18] It is also imperative to map the genetic spectrum of thalassemia, particularly in regions where common mutation or deletions are not well understood.[13] However, in countries such as Indonesia, where cost and accessibility pose significant barriers, an alternative screening strategy is necessary. In primary health care (puskesmas) or puskesmas auxilary, a combination of hematological examinations and peripheral blood smear analysis can be employed to assess thalassemia risk, with automated blood cell counters providing quantitative data on hemoglobin (Hb) levels, MCV, MCH, and red blood cell counts.

Peripheral blood smear analysis can reveal signs of microcytosis, hypochromia, and other morphological abnormalities. However, should any hematological parameter deviate from established norms, specifically Hb <11 g/dL, MCV <80 fL, or MCH <27 pg, referral to a higher-level healthcare facility is warranted for further evaluation.[1]

Although MCV and MCH are accepted as first-line screening parameters for identifying beta-thalassemia carriers for screening in resource-limited setting according to the Indonesian Ministry of Health, Sahiratmadja et al. have reported that MCV <80 fL and/or MCH <27 pg demonstrated relatively low sensitivity (38.6%) and specificity (67.6%) in sample from family members of patient with thalassemia major, when compared to diagnoses based on HbA_2 levels. This finding underscores the inadequacy of relying solely on erythrocyte indices for diagnosing beta-thalassemia, thereby highlighting the necessity of incorporating hemoglobin analysis or DNA examination for accurate diagnosis.

Various indices have been widely used as preliminary screening tools to identify potential beta-thalassemia carriers and distinguish between thalassemia traits and iron deficiency anemia (Table 5).[19]

Table 5. Erythrocyte/ discriminant indices for preliminary beta-thalassemia carrier screening.

Erythrocyte Indices	Calculation	Cut-off value for BTT
Mentzer	MCV/RBC	≤ 13
England & Fraser	MCV - (5 x Hb) – RBC – 3.4	≤ 0
Shine & Lal	MCV x MCV x MCH/100	≤ 1530
Srivastav	MCH/RBC	≤ 3.8
Sirdah	MCV – RBC – (3 x Hb)	≤ 27
Ehsani	MCV – (10 x RBC)	≤ 17
Green-King	(MCV x MCV x RDW) / (Hb x 100)	≤ 65
Matos & Carvalho	(1.91 x RBC) + (0.44 x MCHC)	> 23.85
RDW Index	MCV x RDW / RBC	≤ 220

RBC: Red Blood Cell, RDW: Red Cell Distribution Width, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration

To provide better diagnostic value, Salim et al. suggested new cut-off points for Mentzer, RDW, Green King, and Sirdah with values of 13.44, 233.4, 75.06, and 35.52, respectively.[10]

All studies highlight the significant challenge of diagnosing BTT in populations where iron deficiency and thalassemia coexist, especially in regions with high BTT prevalence, such as Indonesia. One study revealed a striking discrepancy between anemia diagnosis via finger-prick testing (86.7%) and confirmation through CBC (21.9%), demonstrating the limitations of simple screening tools in clinical practice.[9]

Variations in methodological approaches across different studies may lead to discrepancies in the results reported. The number of participants in these studies varied from 23 to 223, affecting the statistical strength of the findings. Additionally, all studies employed hemoglobin analysis as a confirmatory test to accurately diagnose BTT, with one study also utilizing DNA sequencing. This indicates that while erythrocyte indices are useful for initial screening, hemoglobin analysis is essential for confirming the diagnosis, and molecular diagnostics can be used as an additional method for a definitive diagnosis.

The efficacy of erythrocyte indices appears to differ across various regions and populations. Among these indices, the Green-King Index generally exhibits the highest sensitivity. In one study, the Shine & Lal Index demonstrated excellent sensitivity at 96%, yet its specificity was relatively low at 40.5%, thereby limiting its utility in populations where BTT is prevalent.[13] The Mentzer Index yielded mixed results, displaying moderate sensitivity but high specificity, which makes it valuable for excluding

BTT, particularly in regions with high incidences of iron deficiency.[10–13] It is essential to differentiate IDA from BTT to avoid unnecessary iron treatment for beta-thalassemia carriers.

Despite variations in diagnostic effectiveness across different populations and methods, erythrocyte indices remain a cost-effective screening option for BTT. Future studies should prioritize the development of population-specific cut-off values and enhance access to hemoglobin electrophoresis in resource-limited settings. These advancements will improve the accuracy of screening programs and facilitate clinical decision-making for individuals suspected of being thalassemia carriers, thereby contributing to thalassemia prevention efforts.

This review synthesizes erythrocyte indices for the screening of beta-thalassemia trait in Indonesia, utilizing multiple databases and concentrating on laboratory indices pertinent to clinical practice. However, there are several limitations. Only six studies met the criteria for inclusion, and no formal quality appraisal was conducted. Additionally, the possibility of publication bias cannot be excluded, and the findings may have limited generalizability beyond the Indonesian context.

5. Conclusion

Research indicates that erythrocyte indices serve as valuable preliminary screening tools for identifying beta-thalassemia carriers; however, their diagnostic efficacy varies considerably depending on population characteristics, disease prevalence, and local laboratory methodologies. Among the indices assessed, the Green–King Index demonstrated the highest reliability, although this finding requires further study. Nonetheless, hemoglobin electrophoresis remains indispensable for the definitive identification of beta-thalassemia carriers in Indonesia. The utilization of this method as a confirmatory test is crucial to ensure diagnostic precision and to address the limitations associated with erythrocyte index-based screening.

Ethical Approval

There is no ethical approval

Conflicts of Interest

The authors declare no conflict of interest.

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Author Contributions

Conceptualization, IM and WEN; methodology, IM, WEN and AVRM; validation, AVRM; data curation, IM, ARK, AAH; writing-original draft preparation, IM, ARK; writing-review and editing, IM, ARK, WEN, AAH, AVRM; visualization, IM.

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