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Early screening diagnostic test in adolescents with a family history of thalassemia

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ABSTRACT

Background: Thalassemia is a major public health concern in Indonesia, particularly in families with a known history of the ailment. This study aimed to assess the validity, reliability, sensitivity, and specificity of a thalassemia screening instrument that included a questionnaire and a scoring model.

Methods: A cross-sectional study was conducted in Tegal, Central Java (November 2024–April 2025), involving 64 adolescents aged 10–24 years from extended families with a history of thalassemia, selected through purposive sampling. Inclusion criteria included willingness to participate, ability to communicate, and signed informed consent, while exclusion criteria included incomplete participation, other blood disorders, medical complications, or withdrawal. Screening consisted of a structured questionnaire and physical examination (score 0–8), followed by CBC and HbA2 testing. Statistical analyses included the Kolmogorov–Smirnov test, chi-square tests, Pearson or Spearman correlations, and diagnostic accuracy measures (sensitivity, specificity, PPV, NPV) using a 2×2 table.

Results: The screening instrument showed a sensitivity of 76.5%, a specificity of 85.6%, and an AUC of 0.76. It demonstrated acceptable internal consistency and significant construct validity, indicating moderate diagnostic accuracy for identifying thalassemia trait.

Conclusion: The screening tool established in this study was appropriate for teenagers with a family history of thalassemia and had modest diagnostic performance. Integration into existing public health systems is both achievable and recommended.

Keywords: adolescents, diagnostic test, early screening, screening instrument, thalassemia.
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INTRODUCTION

Thalassemia is a genetically inherited hematological condition that has serious clinical and economic consequences, particularly in low- and middle-income nations. The rise in the incidence of serious thalassemia in Indonesia is mostly owing to a failure to find thalassemia gene carriers early enough. The goal of this study was to create a low-cost, user-friendly screening tool that could be implemented into the public health system to improve early detection.[1,2] According to data from the Indonesian Ministry of Health, more than 10,500 cases of thalassemia major were reported in 2022, with the majority detected after symptoms manifested due to a lack of comprehensive screening among teenagers, particularly those with a family history.[3,4] According to Rujito et al., standard premarital screening is frequently useless since many couples are already well into the wedding planning process before knowing their carrier status. Early screening for high-risk groups is therefore critical.[5]

Programs that prioritize screening and early detection in high-risk populations have been proven effective in reducing the birth of children with thalassemia major throughout Southeast Asia. In Singapore, more than 20 years of national screening programs have successfully reduced thalassemia major births to only three cases. Similarly, Thailand's 13-year program has reduced the birth rate to 5.4 per 1,000 births. Cost analysis shows that treating thalassemia can be up to 72 times more expensive than screening.[6,7,8] The WHO also estimates that the annual cost of a national prevention program is similar to the cost of treating a thalassemia major patient for a year, pointing out that prevention costs remain relatively steady while treatment expenses increase.[9]

Prevention of thalassemia is a practical and effective strategy to reduce the significant burden experienced by affected children, families, and countries. Raising awareness among high-risk communities about the prevalence, impact, and management of thalassemia is crucial.[10,11] Family history screening usually begins with the index case, a child with thalassemia major, and extends to three generations to map inheritance patterns and identify potential gene carriers.[12,13] Individuals with a family history of thalassemia, including parents,

siblings, and distant relatives of affected children, are at high risk due to the autosomal recessive inheritance of this disease. Therefore, comprehensive and structured genetic counseling is necessary to detect carrier status in extended families.[14] Although initial identification can be done through family history and routine hematology indices (MCV and MCH), definitive diagnosis requires hemoglobin analysis, such as hemoglobin electrophoresis or DNA testing, to account for other mutation variations that may mimic thalassemia.[15,16]

Many studies have shown that thalassemia screening programs are far more effective than curative approaches. Screening can be applied to the general population as well as high-risk groups; however, there are currently no specific guidelines targeting high-risk families. Research from Pakistan reports that approximately 62.2% of extended family members of individuals with thalassemia are beta-thalassemia carriers.[17] Meanwhile, a 2016 study in India reported a prevalence of thalassemia carriers of 21.9% in families with members with thalassemia, significantly higher than the 5–8% rate in the general population. These findings suggest that family-based screening is a more practical and cost-effective strategy, especially in resource-limited settings.[18,19]

Early screening during adolescence is crucial for identifying thalassemia carriers before they enter the reproductive stage, such as marriage and pregnancy. Effective screening must be affordable, simple, non-invasive, and demonstrate high validity and reliability. In areas with limited resources, primary health workers play a key role in expanding screening coverage. Banyumas, which has the highest number of thalassemia patients in Central Java, has implemented routine thalassemia screening since 2022. On the other hand, interviews with the Non-Communicable Disease Control Program in Tegal show that thalassemia screening is still unstructured and conducted passively in conjunction with adolescent anemia programs, without integration into adolescent health center activities.

A preliminary study of the thalassemia one day care (ODC) Unit at Kardinah Regional Hospital, the sole ODC covering the Tegal and Brebes districts, revealed 36 children with thalassemia major who got blood transfusions twice a week. The purpose of this study is to compare the validity, reliability, sensitivity, and specificity of thalassemia screening tools (medical history forms and simple physical examinations) to HbA2 tests (Hb electrophoresis, the gold standard) for early detection of thalassemia gene carriers in adolescents aged 10-24 years with a family history of thalassemia. These findings will help to improve secondary and tertiary preventive efforts and increase early detection coverage in Indonesia's high-risk groups.

METHODS

This cross-sectional study was carried out in Tegal from November 2024 to April 2025 and included 64 teenagers aged 18-24 years from big families with a history of thalassemia who were chosen using purposive sampling. A screening prototype was built and verified by field testing, with the HbA2 blood test serving as the gold standard. The research techniques included a screening questionnaire (based on the Mentzer Index and familial risk data), a complete blood count (CBC), and a HbA2 measurement. A pilot study was carried out prior to full adoption.

Inclusion criteria included adolescents aged 10–24 years from families with thalassemia, willingness to participate in all research procedures, ability to communicate effectively, and provision of informed consent. Exclusion criteria were incomplete participation, other blood disorders, medical complications, or voluntary withdrawal.

Data collecting methods include written agreement, screening instruments (anamnesis and physical examination), and laboratory testing (CBC and HbA2). The screening methodology consists of a structured medical history that covers family history, fatigue, transfusion history, and illness susceptibility, as well as a physical examination that evaluates conjunctival and facial pallor and growth retardation. The grading system goes from 0 to 8: 0-2 suggests low risk, 3-4 indicates moderate risk, 5-6 shows high risk needing CBC and reticulocyte testing, and 7-8 indicates extremely high risk requiring HbA2 testing and genetic counseling.

A two-by-two contingency table was used to calculate diagnostic accuracy (sensitivity, specificity, PPV, and NPV). Pearson's correlation and Cronbach's alpha were used to assess construct validity and internal consistency, respectively. Additional analyses included chi-square tests for categorical variables, Kolmogorov-Smirnov tests for normality, descriptive statistics (mean ± SD, median, and IQR), and Pearson/Spearman correlations for continuous data. Data were analyzed using SPSS version 26.0, with a significance level of $p < 0.05$.

RESULTS

Early diagnosis of thalassemia carriers is critical because most teenagers carrying the trait are asymptomatic and can only be found through screening. Knowing their carrier status allows people to make more informed reproductive decisions and helps prevent the birth of children with thalassemia major. Secondary prevention techniques, such as community and school-based screening programs developed in various countries, have been beneficial since they target teenagers before they marry or have children.[20,21]

This study examined the characteristics of respondents based on their age and gender, with participants drawn from the extended relatives of thalassemia patients. The screening process comprised a medical history, physical examination, complete blood count, and HbA2 analysis. As stated in **Table 1**, the majority of respondents were late teenagers (18-21 years old; 31.25%) and male (53.12%). Diagnostic tests were undertaken to assess the tool's capacity to discriminate persons with and without thalassemia characteristics, with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) as the primary metrics.

Table 1 and **2** demonstrate the screening model's usefulness for early detection in situations with limited diagnostic resources and a high thalassemia burden. A sensitivity of 75.6% implies that around three-quarters of thalassemia carriers were correctly detected, whilst a specificity of 82.5% suggests that the majority of non-carriers were correctly classified without false positives. The ROC curve also shows

strong diagnostic performance, with 75.60% sensitivity and 86.20% specificity (**Figure 1**).

The ROC curve is constructed at various thresholds to differentiate between positive and negative cases, demonstrating the link between sensitivity and false positive rate (1-specificity). A curve closer to the upper left corner suggests better test performance, and in this study, the ROC curve was higher than the diagonal reference line, suggesting superior discrimination. The Area Under the Curve (AUC), which measures the model's ability to distinguish between individuals with and without thalassemia characteristics, was around 0.76, indicating a reasonably good diagnostic tool. A higher AUC value indicates stronger discriminating between positive and negative situations.

The screening tool demonstrated good diagnostic performance, with a sensitivity of 75.60% for detecting thalassemia carriers and a specificity of 86.20% for identifying healthy individuals. An AUC value of approximately 0.76 indicates acceptable accuracy and reliability. Using a threshold score of ≥6 on combined medical history and physical examination, participants are advised to undergo CBC and Hb analysis. This tool is suitable for early screening prior to confirmation by Hb electrophoresis or molecular testing.

DISCUSSION

The combination of a family-based approach and a proven screening technique creates a proactive strategy for detecting thalassemia carriers before marriage or pregnancy. This study demonstrates that semi-quantitative approaches, a brief medical history, and a physical examination can yield enough accuracy when the gold standard diagnostic test is unavailable. This methodology can be duplicated and tailored to address different genetic disorders and public health concerns. It was created utilizing a family-community framework that included early clinical screening, the Mentzer Index, and HbA2 testing as the gold standard for confirmation.[22,23]

This strategy employs the Mentzer Index as an efficient and cost-effective first screening technique, followed by confirmatory testing for probable cases

Table 1. Frequency distribution of respondent characteristics according to age and gender

Characteristics	Frequency (n=64)	Percentage (%)
Age, years		
10-13	12	21.42
14-17	18	28.13
18-21	20	31.25
22-24	14	25
Gender		
Male	34	53.12
Female	30	46.88

Table 2. Analysis of examination results using tools for haemoglobin A2 (HbA2) examination in thalassemia screening.

	HbA2 examination (*gold standard*)		
Screening instruments	Positive	Negative	Number
Positive	31	5	36
Negative	10	18	28
Total	41	23	64
Sensitivity	75.60%		
Specificity	82.60%		
Positive predictive value (PPV)	86.10%		
Negative predictive value (NPV)	64.30%		

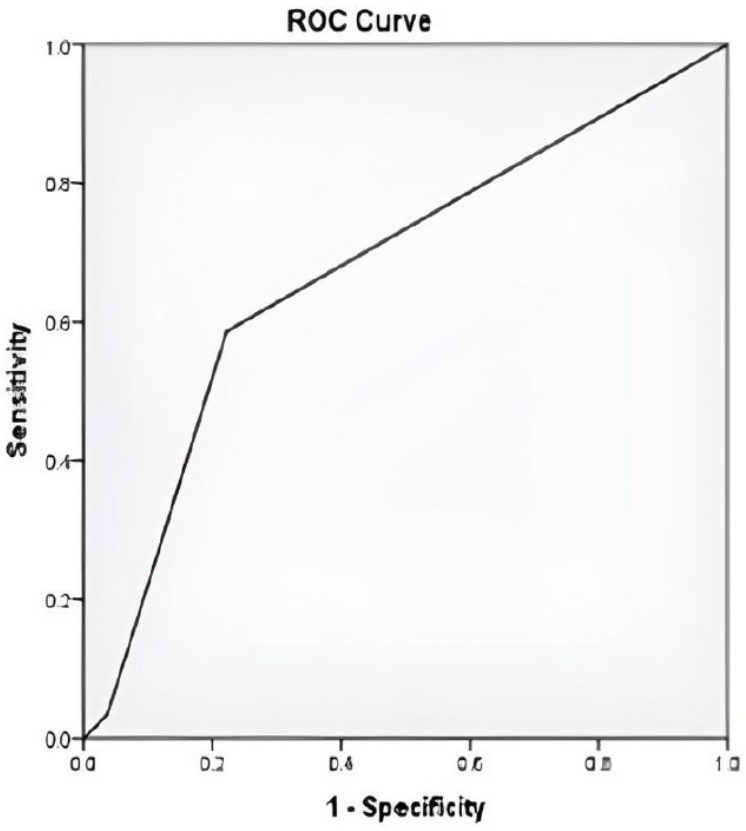

Figure 1. Receiver operating characteristic (ROC) curve evaluating the accuracy of haemoglobin level in identifying thalassemia status.

using hemoglobin electrophoresis or HPLC. The Mentzer Index, which has been verified in multiple populations, offers a high sensitivity for discriminating thalassemia from iron deficiency anemia. The diagnostic evaluation demonstrates that this model has acceptable accuracy, sensitivity, and specificity to support community-level screening. These findings highlight the importance of simple hematological markers when used with a structured, risk-based screening approach.[24,25]

Sensitivity measures the tool's capacity to detect carriers of thalassemia characteristics. A result of 75.6% suggests that early screening can identify nearly three out of every four carriers. Rujito et al. found a similar sensitivity of 71.1% for the Mentzer Index in the Indonesian population, confirming the validity of these findings in the local context. Although most adolescents with the characteristic are identified, the probability of false negative results remains around 24.4%.[26]

Specificity refers to the tool's capacity to correctly identify individuals who do not have thalassemia characteristics (true negatives). A specificity of 82.5% is regarded good, implying that most people without thalassemia characteristics are unlikely to have false positive results. This finding is congruent with that of Ghafoor et al. (2016), who reported a 79% specificity among Pakistani teenagers tested using a similar hematological index. A PPV of 68.2% suggests a moderate possibility that individuals with positive screening findings genuinely carry the trait, implying that around three out of every ten positive results may be false positives, as expected in populations with moderate to low carrier prevalence. Therefore, all positive screening results should be confirmed with HbA2 or hemoglobin electrophoresis testing. The NPV of 94.8% indicates a high probability that individuals with negative results are truly non-carriers, exceeding the NPV of 91.2% reported by Mahmood et al. in Bangladeshi adolescents.[19]

Family-based approaches such as tiered screening, which targets siblings and relatives of index thalassemia patients, have proven to be highly effective and cost-efficient; a study in Pakistan identified 31% of carriers within one family. Although Indonesia has a high burden of thalassemia, national systematic screening guidelines, especially for children, are still limited. In resource-limited settings, expanded family-based screening represents a practical and strategic approach.[10,11]

Early detection of thalassemia carriers is a critical component of national prevention efforts, especially in areas with limited laboratory capacity and health resources. Screening usually begins with peripheral blood tests and hemoglobin electrophoresis, with DNA analysis used for confirmation if necessary.[10,27] Screening should be conducted before marriage or pregnancy, and adolescents are encouraged to find out their carrier status as early as possible. A family-based approach is essential, starting with Ring I (immediate family members of thalassemia patients) and expanding to the wider family, to support early detection and premarital screening, and ultimately help achieve zero births of major thalassemia.[28,29]

Adolescents who test negative can be safely excluded from further assessment, making this screening strategy efficient for early detection. In families affected by thalassemia, adolescents may experience a higher sense of vulnerability, which may increase their participation in screening programs. This model also integrates encouragement to act through school-based education and peer involvement, promoting voluntary participation. Additionally, the simplicity of this assessment reduces barriers by enabling early detection without invasive or costly procedures. As emphasized by Modell and Darlison (2008), resource-limited settings are in dire need of practical early screening models, and this approach supports risk stratification without relying solely on laboratory diagnostics.[22]

The model's sensitivity (76.5%) and adequate specificity (86.2%) make it helpful as an initial screening tool for detecting thalassemia carriers in high-risk adolescents. The simple anamnesis and physical examination components are comparable with prior validation investigations, which found that pallor, growth retardation, and weariness were common clinical signs.[30] The reliance on non-laboratory characteristics is also consistent with WHO guidelines for genetic screening in resource-limited settings. This study provides a practical instrument to help the Indonesian Ministry of Health implement its national genetic disorder control strategy. These findings illustrate the potential of low-cost, evidence-based innovations to solve persistent public health concerns in underdeveloped countries. This scoring system, which combines a family- and community-based approach with a validated screening model, offers a proactive strategy for detecting thalassemia carriers prior to marriage or pregnancy while staying easy enough for non-specialists to utilize.

This study demonstrates that a semi-quantitative technique that combines medical history and physical indicators can yield enough accuracy when the gold standard diagnostic method is unavailable. The screening technique described here can be applied to additional hereditary disorders and public health concerns. Although beneficial, this technique should be used with caution and always followed by confirmation testing with more accurate methods, such as HbA2 testing. To boost detection accuracy, screening systems must be enhanced with new methods and supportive policies. It is appropriate as an initial screening step, particularly in resource-limited settings, because it is a simple and low-cost technology in comparison to Hb electrophoresis or DNA analysis.

The study has major limitations. The small sample size and deliberate sampling from big families with known thalassemia burden limit the generalizability of the findings to a larger teenage cohort. The cross-sectional design prevents evaluation of the predictive performance of the tool over time. The screening tool was only validated against the HbA2 test, without confirmation methods such as hemoglobin electrophoresis or molecular analysis, which may affect diagnostic accuracy. Reliance on self-reported history and basic physical examination may have introduced reporting or examiner bias. Additionally, moderate sensitivity and AUC suggest that some carriers may remain undetected. Future studies should include larger and more diverse samples, incorporate molecular confirmation, and evaluate longitudinal performance to strengthen accuracy and public health applicability.

CONCLUSION

This study shows that the early thalassemia screening model works well in distinguishing individuals who have and do not have thalassemia traits. This tool demonstrates consistent ability in identifying thalassemia gene carriers while accurately ruling out non-carriers, supported by diagnostic curves that show good overall discrimination. Its simple components, medical history, physical examination, and basic hematological assessment, make it practical and suitable for early screening, especially in settings with limited diagnostic resources. Integrating this model into existing public health systems is a feasible and recommended step to facilitate early detection and timely confirmatory testing.

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ETHICAL CONSIDERATION

The Human Research Ethics Committee of the Faculty of Public Health, Universitas Diponegoro, Indonesia approved the study (No. 2023-20034-50, November 25, 2023). All participants provided written consent.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

AUTHOR CONTRIBUTIONS

NF designed the study, developed the methodology, and drafted the manuscript. AK contributed to the study design, data management, and supervision. SAN participated in data analysis and interpretation. AM assisted in validation, resource provision, and critical review of the manuscript. FH supported statistical analysis, visualization, and final editing. All authors have read and approved the final manuscript.

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APPENDIX

THALASSEMIA SCREENING FORM

RESPONDENT CHARACTERISTICS

No.	Respondent Number	Answer
1.	Name/Initials	
2.	Age (date of birth)	
3.	Address	
4.	Last education	a. Elementary school b. Junior high school c. Senior high school d. College
5.	Work	a. Self-employed b. civil servant c. Housewife d. Private employees e. Others……..

HISTORY OF THALLASEMIA

Answer the questions below according to your condition!

No.	Question	Answer
1.	Has anyone in your family ever been diagnosed with thalassemia or another blood disease?	
2.	Has anyone in your family ever been diagnosed with anemia or other blood problems?	
3.	Which family members are known to have thalassemia?	
4.	What type of thalassemia does the family member suffer from?	
5.	What is the health condition of family members who have thalassemia?	
6.	Has any family member ever had a blood transfusion or other treatment related to thalassemia?	
7.	Have any family members had genetic testing or consultations with a geneticist regarding thalassemia?	

SYMPTOMS AND SIGNS

No.	Statement	Answer	
		Yes	No
1.	I often feel tired or weak.		
2.	I often experience dizziness or shortness of breath.		
3.	I have experienced frequent drowsiness or difficulty concentrating.		
4.	I have experienced pain in my body without any clear cause.		
5.	I often feel pale or my skin looks pale.		
6	I often experience pain		

No.	Question	Answer	
		Yes	No
EXAMINATION HISTORY			
1.	Are there any signs of anemia such as pale skin, pale lips, or physical weakness?		
PERSONAL MEDICAL HISTORY			
1	Have you ever had a blood test before? If yes, what was the result?.....		
2.	Do you have a history of chronic illness or other blood disorders?		
BLOOD TRANSFUSION HISTORY			
1.	Have you ever received a blood transfusion before? If yes, how often and for what conditions? ……		
2.	Do you know what type of blood you received during a transfusion?		
3.	Have you ever had blood test results that showed low hemoglobin levels or low red blood cell counts?		
PHYSICAL EXAMINATION			
1	Height (cm)		
2	Body weight (kg)		
3	Eye: a. Conjunctiva (anemic/not) b. Sclera (icteric/not)		
PHYSICAL EXAMINATION			
4	Palms and soles (pale/not)		
5	Changes in facial bones (snub nose)		
6	Signs of puberty in girls a. Menstruation b. Breast enlargement Signs of male puberty* a. Wet dream b. Testicular enlargement		
7	Pulse		
8	Blood pressure		
BLOOD TEST			
1	Hemoglobin (anemia/not)		
2	Mean corpuscular volume (MCV)		
3	Mean corpuscular hemoglobin (MCH)		
4	Red cell distribution width (RDW)		
6	Hemoglobin (Hb) analysis		