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Integrated Approach to Improve Hemoglobin in Adolescent Girls: A Quasi-Experimental Controlled Study

Dewi Erna Marisa[1*], Erida Fadila[1], Ahmad Syaripudin[1], Hasril Desiathul Hamdani[1], Lily Wahyuni[1], Tantri Maulani Putri[1]
[1]Institute Teknologi dan Kesehatan Mahardika, Cirebon, West Java, Indonesia

***Corresponding Author**: E-mail: dewi.erna@mahardika.ac.id

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ABSTRACT

Introduction: A major public health issue affecting adolescent girls is anemia, largely as a result of insufficient iron intake, menstrual blood loss, and poor nutritional literacy. A single-component intervention either supplementation or education rarely produces more than a modest amelioration. Evaluating the effectiveness rates of an integrated intervention, consisting of an iron-rich diet as a component of the regimen, iron supplementation as supplementary factor, and structured nutritional education versus single-component strategies aimed to improve hemoglobin (Hb) among adolescent girls, this study aims to address limitations associated with a single-component intervention approach alone.
Methods: A quasi-experimental controlled non-randomized design with prospective follow-up was carried out with 180 adolescent girls between 12–18 years of age recruited from a stratified sampling. Participants were recruited in school and divided into three intervention arms to reduce contamination. In addition to the oral iron supplementation (60 mg, two times weekly for 12 weeks), the intervention utilized iron-rich dietary intervention, and included six structured education sessions based on the Health Belief Model. Hemoglobin levels were measured at baseline, Month 3, and Month 6 using the cyanmethemoglobin method. Dietary behaviour and adherence were assessed on the basis of validated instruments. Data were analyzed using repeated measures ANOVA and assumption diagnostics with appropriate revisions, Tukey post-hoc comparisons and multivariate linear regression adjusted for baseline Hb and all relevant covariates. Statistical significance was defined as $p < 0.05$.
Results: No differences between baseline hemoglobin levels ($p = 0.632$). All intervention arms showed significant increases of Hb over six months (time effect, $p < 0.001$). The integrated intervention achieved the most mean improvement (3.5 g/dL) than supplementation plus education (2.2 g/dL) and diet plus education (1.6 g/dL) and there were significant ($p < 0.001$) between-group differences. Intervention type and adherence level were still significant predictors of hemoglobin improvement in adjusted regression analysis.
Conclusion: The integrated intervention showed superior comparative efficacy compared with single-component approaches in improving hemoglobin levels among adolescent girls. The recommendations of this study are supported by evidence based on evidence-based, multi-component, and structured in school prevention and control programs in the development of adolescent health policies.

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INTRODUCTION

Anemia is a major public health crisis globally and women are disproportionately affected by anemia. Globally, nearly 41.5% of girls from 10 to 18 years of age in low- and middle-income countries (LMICs) have anemia (1) most commonly due to insufficient consumption of iron and greater physiological demand during puberty. In Indonesia, the prevalence is up to 37%, which is a reflection of the continued nutritional inequalities and restricted access to preventive health care services (2,3).

Adolescence represents a critical developmental period characterized by rapid somatic growth; cognitive maturation; and hormonal transitions, all of which substantially increase micronutrient requirements, particularly iron (4). Due to dietary inadequacy, low rates of nutrition literacy, and poor health- seeking behaviors, adolescent girls have heightened susceptibility to such risks as iron deficiency in their daily lives. After normal menstruation comes the requirement of iron, so a heavy risk for anemia occurs, especially between the physiological requirements and what intake may or may not satisfy (5,6).

Adolescent iron deficiency anemia has a long-established impact, which results in poor endurance, decreased focus, poor achievement in school and impairment of neurocognitive development (7,8, 9,10). Anemia in this population is often attributed to iron deficiency however, hemoglobin concentration alone may fail to distinguish a possible etiological factor from inflammation or a genetic etiology. However, low levels of hemoglobin remain a clinically relevant marker of hematologic vulnerability in adolescent populations.

The problem is not addressed by different interventional strategies even though the burden of anemia among Indonesian adolescents is significant. Current programs often focus mainly on one modality, either iron supplementation or health education, rather than including complementary nutritional and behavioral modalities. Some previously published studies identified only modest benefits from supplementation or dietary modification on its own and relatively limited evidence from the comparative point of view of multi-component interventions within the same analytic framework. Furthermore, very limited studies in LMIC schools have systematically assessed combined biological (diet and supplementation) and behavioral (education driven adherence) strategies over a longitudinal follow up period.

To fill the current gap, this study aims to explore the comparative effect of an intervention combining an iron-rich diet, iron supplementation, and organized nutritional education on the blood hemoglobin level among adolescent girls. This study employed a quasi-experimental controlled non-randomized design with prospective follow-up to compare three intervention arms within a school-clustered context over six months. The study adds to the evidence regarding the efficacy of integrated anemia prevention on adolescent girls by including both biological and behavioral factors within a structured comparative framework which adds further evidence in the literature. The intervention was designed as a structured multi-component program that integrated intermittent iron supplementation (60 mg twice weekly), dietary changes, and behavioral-based nutritional education targeting both biological determinants as well as behavioral ones.

METHOD

Research Type

This study adopts a quasi-experimental controlled non-randomized design and prospective follow-up to test the effectiveness of three intervention strategies, an iron-rich diet, iron supplementation, and nutritional education, on hemoglobin (Hb) levels among adolescent girls at risk of anemia. To minimize cross-participant contamination across students in the same academic setting, the participants were divided into three intervention arms on the basis of school-level allocation. Randomisation and allocation concealment were not conducted in individual participants for reasons of ease of operation. A causal inference should be interpreted with caution in view of these practical problems. Since allocation was cluster based (for each intervention arm school was only one school being given randomly assigned with a single intervention arm), there was no full allowance for the possibility for intra-cluster correlation. The research covered a period of 6 months (January–June 2024) in three junior and senior high schools in Cirebon, West Java, Indonesia. Baseline equivalence between groups was examined by one-way ANOVA for continuous variables and chi-square for categorical variables including age, BMI, menstrual history and baseline hemoglobin level.

Population and Sample/Informants

The target population was adolescent girls ages 12–18 identified as likely to be at risk for anemia based on preliminary hemoglobin screening. Sample size was calculated a priori using repeated-measures ANOVA (within–between interaction) in G*Power version 3.1.9.7.

Parameters included:

Effect size f = 0.30

$\alpha = 0.05$

Power $(1-\beta) = 0.80$

Number of groups = 3

Number of measurements = 3

Assumed within-subject correlation (ρ) = 0.50

Nonsphericity correction (ε) = 1

The calculation was cross-verified using the approximation:

$$n = \frac{2(Z_{\alpha/2} + Z_{\beta})^2}{f^2}$$

With $Z\alpha/2 = 1.96$, $Z\beta = 0.84$, and f = 0.30, yielding a minimum total sample size of 174 participants. Accounting for 20% attrition, the final sample included 180 participants (n = 60 per group).

Clustering effects were not conventionally modeled in the power calculation, and consequently, variances are to be conservative and not assumed.

Inclusion criteria:

Female adolescents aged 12–18 years

Mild to moderate anemia (Hb 10–11.9 g/dL)

Not receiving iron therapy prior to the study

Willing to participate and signed parental/guardian informed consent

Exclusion criteria

Diagnosed chronic illnesses (e.g., thalassemia, renal disease)

Severe anemia (Hb < 10 g/dL)

Incomplete follow-up data

Research Location

The study was conducted in 2024 in three junior and senior high schools in Cirebon, West Java, Indonesia.

Instrumentation or Tools

Three instruments were employed for data collection:

Hemoglobin Measurement: Hemoglobin levels were measured using the cyanmethemoglobin method at three time points: baseline (Month 0), Month 3, and Month 6. All procedures followed standard laboratory protocols for accuracy and reliability.

Nutritional Behavior Questionnaire: A validated food frequency questionnaire (FFQ) assessed:

Frequency of consumption of iron-rich foods

Adherence to supplementation

Dietary patterns supporting iron absorption

The FFQ demonstrated strong psychometric properties with a Content Validity Index (CVI) of 0.89 and reliability coefficient (Cronbach's alpha) of 0.82.

Supplement Adherence Checklist: A structured adherence checklist assessed weekly compliance, using pill-counting and 7-day recall methods. Reliability testing yielded Cronbach's alpha of 0.86.

Intervention Protocol

The intervention consisted of three structured components iron supplementation, dietary modification, and nutritional education implemented over a six-month study period. Participants in the supplementation groups received oral iron tablets containing 60 mg elemental iron per dose, administered twice weekly on non-consecutive days for 12 weeks. The supplementation was provided under supervised administration at school, and adherence was monitored using pill counts and a 7-day recall log. Participants were instructed to take the supplement after meals and avoid tea or coffee within one hour to optimize absorption.

The dietary intervention focused on increasing the intake of iron-rich foods and improving iron bioavailability. Participants were encouraged to consume heme iron sources 3–4 times per week, include non-heme iron sources in daily meals, and combine intake with vitamin C-rich foods, while reducing inhibitors such as tea and coffee. Dietary adherence was assessed using a validated Food Frequency Questionnaire and monitored weekly.
Nutritional education was delivered through six structured sessions (45–60 minutes each) over three months, based on the Health Belief Model. The sessions addressed anemia risk, consequences, benefits of iron intake, barriers to adherence, and self-efficacy, using interactive methods such as discussions and practical demonstrations. Attendance was recorded to ensure fidelity. Participants in the integrated group received all three components simultaneously, while comparison groups received combinations of two components according to the study design.

Data Collection Procedures

Data collection was carried out over a six-month period using structured and standardized procedures to ensure consistency and accuracy. At baseline, demographic information, anthropometric measurements, and hemoglobin levels were collected, with hemoglobin assessed using the cyanmethemoglobin method performed by certified laboratory personnel. Participants also completed validated questionnaires evaluating dietary behavior and prior supplementation adherence. Throughout the intervention period, weekly monitoring was conducted to document dietary compliance, supplement intake, and participation in nutritional education sessions. During the intervention period, iron supplementation (60 mg twice weekly for 12 weeks) was administered under supervision, while dietary adherence and participation in six structured educational sessions were monitored weekly. A midline hemoglobin assessment was performed at Month 3 to measure interim progress. At the conclusion of Month 6, final hemoglobin measurements and comprehensive dietary behavior evaluations were repeated following identical protocols to maintain measurement reliability. All collected data underwent double entry, cross-verification by the research team, and secure digital storage to ensure data integrity and overall quality.

Data Analysis

Data were analyzed using SPSS (version XX). Descriptive statistics were calculated to summarize baseline characteristics. For longitudinal analysis, a mixed-design repeated-measures ANOVA was conducted with:

Within-subject factor: time (baseline, Month 3, Month 6)

Between-subject factor: intervention group (3 levels)

Normality was assessed using the Kolmogorov–Smirnov test and homogeneity of variances using Levene's test. Sphericity was evaluated using Mauchly's test; when violated, Greenhouse–Geisser correction was applied. Effect sizes were reported using partial eta squared ($\eta^2 p$), with 95% confidence intervals for mean differences.

Post-hoc comparisons were performed using Tukey's HSD test. Multivariate linear regression (enter method) was conducted to identify predictors of hemoglobin change (Month 6 − baseline). Independent variables included intervention group (dummy-coded), adherence percentage, baseline hemoglobin, age, BMI, and menstrual blood loss score. Multicollinearity was assessed using variance inflation factor (VIF), and model fit was evaluated using adjusted R^2. Because clustering was not explicitly modeled using multilevel techniques, standard errors may be slightly underestimated.

Ethical Approval

Ethical approval for this study was granted by the ITEKES Mahardika Health Research Ethics Committee (Approval No. 175/KEPK.ITEKESMA/V/2024). All participants and their parents/guardians provided written informed consent. Confidentiality, voluntary participation, and the right to withdraw were ensured throughout the study in accordance with the Declaration of Helsinki.

RESULTS

All intervention components, including supervised iron supplementation (60 mg twice weekly), dietary modification, and structured educational sessions, were implemented with high adherence across groups.

Table 1. Descriptive Statistics of Hemoglobin Levels (Baseline, Month 3, Month 6)

Group	Baseline Hb (Mean ± SD)	Month 3 Hb (Mean ± SD)	Month 6 Hb (Mean ± SD)	Total Change (g/dL)
Diet + Education	10.2 ± 0.8	11.0 ± 0.8	11.8 ± 0.9	+1.6
Supplementation + Education	10.1 ± 0.9	11.5 ± 0.9	12.3 ± 1.0	+2.2
Integrated Intervention	10.0 ± 0.7	12.0 ± 0.8	13.5 ± 0.8	+3.5

All groups demonstrated an improvement in hemoglobin levels over the six-month intervention period. The integrated intervention group showed the greatest increase (+3.5 g/dL), indicating superior comparative effectiveness of the combined strategy.

Table 2. Normality and Homogeneity Tests

Variable	Kolmogorov–Smirnov Normality Test (p-value)	Levene's Test for Homogeneity (p-value)	Interpretation
Baseline Hb	0.200	0.412	Normal & homogeneous
Month 3 Hb	0.182	0.365	Normal & homogeneous
Month 6 Hb	0.145	0.298	Normal & homogeneous

All p-values > 0.05 indicate that hemoglobin data were normally distributed with homogeneous variance across groups. These results justify the use of parametric statistical tests, including ANOVA.

Table 3. Repeated-Measures ANOVA for Hemoglobin Change

Source of Variation	F-value	p-value	Conclusion
Within-group change over time (time effect)	87.24	<0.001	Significant improvement over time
Between-group differences (group effect)	45.91	<0.001	Significant differences among interventions
Time × group interaction	39.12	<0.001	Hb improvement varied by intervention type

Mauchly's test indicated a violation of sphericity ($p = 0.031$); therefore, Greenhouse–Geisser correction was applied ($\varepsilon = 0.89$). The time effect demonstrated a large effect size ($\eta^2p = 0.49$), the group effect showed a moderate-to-large effect ($\eta^2p = 0.34$), and the time × group interaction yielded a moderate effect ($\eta^2p = 0.28$). The final model explained 42% of the variance in hemoglobin change (Adjusted $R^2 = 0.42$).

Table 4. Tukey Post-hoc Analysis (Pairwise Comparisons)

Group Comparison	Mean Difference (g/dL)	p-value	Interpretation
Diet + Education vs Supplementation + Education	-0.6	0.032	Significant difference
Diet + Education vs Integrated Intervention	-1.9	<0.001	Highly significant difference
Supplementation + Education vs Integrated Intervention	-1.3	<0.001	Highly significant difference

The integrated intervention group showed significantly greater improvements than both single-intervention groups. Supplementation + education was also significantly more effective than diet + education alone.

Table 5. Multivariate Linear Regression Predicting Hb Change (Month 6 – Baseline)

Variable	β	SE	95% CI	p-value
Integrated intervention	1.45	0.21	1.03–1.87	<0.001
Supplementation + Education	0.76	0.19	0.38–1.14	<0.001
Adherence (%)	0.03	0.01	0.01–0.05	0.002
Baseline Hb	-0.41	0.09	-0.58 – -0.24	<0.001

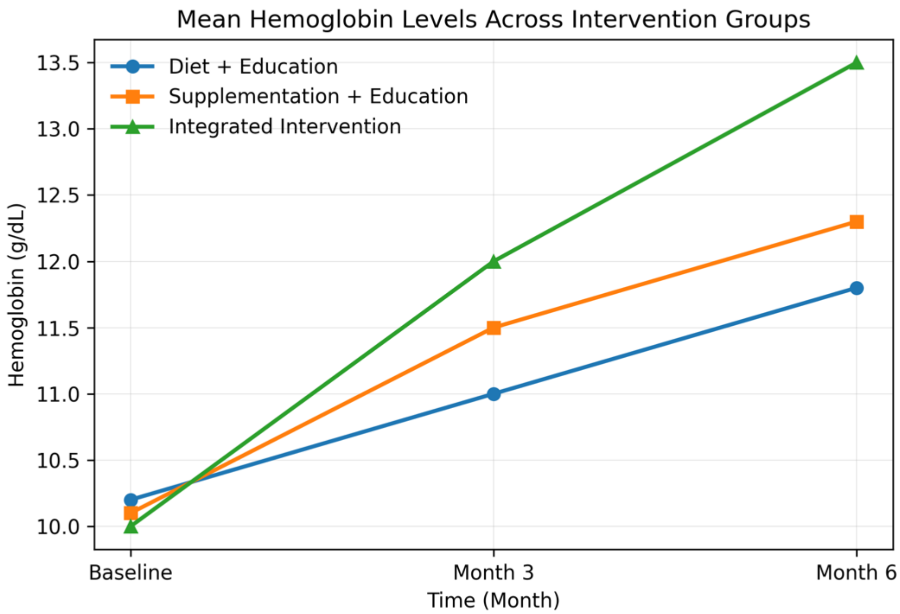

Figure 1. Mean hemoglobin levels across intervention groups at baseline, Month 3, and Month 6

All groups demonstrated an improvement in hemoglobin levels over the six-month intervention period. The integrated intervention group showed the greatest increase (+3.5 g/dL), indicating superior comparative effectiveness of the combined strategy. Figure 1 illustrates the trend of hemoglobin improvement across groups, with the integrated intervention showing the greatest increase over time.

DISCUSSION

Interpretation of Key Findings

The findings indicate hematologic improvement rather than definitive correction of iron deficiency, as ferritin and inflammatory markers were not measured. The findings of this study show that the three intervention groups began with comparable baseline hemoglobin levels, as indicated by the absence of significant differences prior to the intervention ($p = 0.632$). This initial homogeneity confirms that participants entered the study with similar physiological conditions, thereby strengthening internal validity by ensuring that subsequent changes in hemoglobin can be attributed to the interventions rather than pre-existing disparities (11) Over the six-month intervention period, all groups demonstrated meaningful improvements in hemoglobin levels, with increases of 1.6 g/dL in the diet + education group, 2.2 g/dL in the supplementation + education group, and 3.5 g/dL in the integrated intervention group. These results align with prior literature showing that both dietary modification and supplementation are effective strategies for improving hemoglobin in the short term (12,13).

Repeated-measures ANOVA further confirmed that hemoglobin levels increased significantly over time within all groups and that the magnitude of improvement differed significantly across groups ($p < 0.001$). The post-hoc Tukey analysis revealed that the integrated intervention group achieved significantly greater improvements than both the diet + education group and the supplementation + education group, indicating a superior effect of the combined approach. The observed improvement may be attributed to the combined effect of intermittent iron supplementation (60 mg twice weekly), improved dietary intake, and enhanced adherence facilitated through structured education based on behavioral theory (14). Biologically, supplementation provides rapid increases in serum iron, while iron-rich foods supply sustained intake and essential cofactors that support erythropoiesis (15). Educational sessions further enhanced outcomes by improving adherence, correcting misconceptions, and reinforcing consistent supplement intake—behaviors strongly associated with improved hemoglobin levels (16,17). The dietary component, which emphasized vitamin C rich foods and heme iron sources, also contributed to better iron bioavailability and minimized dietary inhibitors (18,19).

These findings are consistent with previous studies showing that single interventions, especially supplementation alone, often produce modest improvements, while combined dietary and educational strategies yield larger gains. The significantly greater increase observed in the integrated group in this study reinforces earlier evidence that multi-component interventions are more effective because they address both biological and behavioral determinants of anemia (20). Collectively, the patterns observed across all statistical analyses including descriptive results, ANOVA, and post-hoc comparisons demonstrate that integrated interventions offer the strongest impact and should be prioritized in adolescent anemia prevention programs.

Comparison with Previous Studies

The findings of this study are consistent with evidence from previous research indicating that integrated, multi-component interventions produce superior improvements in hemoglobin levels compared with single interventions. Prior school-based programs in low- and middle-income countries (LMICs) have shown that iron supplementation alone typically results in moderate gains of approximately 1.5–2.0 g/dL, whereas combined dietary modification and supplementation can achieve increases ranging from 2.8 to 3.6 g/dL—similar to the 3.5 g/dL improvement observed in the integrated group in this study (12,13). These findings reinforce the importance of addressing both physiological and behavioral determinants of anemia. Previous studies have shown that health education significantly increases adherence to supplementation regimens, thereby improving anemia outcome(21,22). Similarly reported that adherence is the strongest predictor of hemoglobin improvement, with participants who maintain consistent supplement intake achieving substantially higher gains than those with low adherence(15). The present study aligns with these conclusions, as the integrated intervention group exhibited the highest levels of dietary compliance and supplement adherence, directly contributing to their greater hemoglobin increase. Furthermore, earlier studies tended to focus on either supplementation or dietary improvement alone, whereas the current study incorporated three complementary strategies diet, supplementation, and structured education simultaneously. This integrated model provides more robust evidence supporting the need for multi-dimensional interventions that can address the complex etiology of adolescent anemia, which encompasses inadequate intake, reduced absorption, menstrual losses, and poor nutritional literacy (23). Collectively, these comparisons highlight that multi-component interventions are consistently more effective than single-modality approaches and underscore the need for comprehensive strategies in reducing anemia among adolescent girls.

Limitations and Cautions

This study has several limitations that warrant careful consideration. First, the assessment of dietary intake and supplementation adherence relied on self-reported questionnaires, which are inherently susceptible to recall and social desirability bias, potentially leading to overestimation of compliance levels (15). Although validated instruments were employed, the absence of biochemical markers such as serum ferritin, transferrin saturation, or soluble transferrin receptor limits the ability to distinguish iron deficiency anemia from other etiologies, as hemoglobin alone is an insufficient indicator of iron status (24). Second, the study was conducted in three schools within a single district, which may limit the generalizability of the findings to populations with different socioeconomic, cultural, and dietary contexts. Third, menstrual blood loss was assessed using a simplified charting method, which may not fully capture intra-individual variability and could introduce residual confounding related to menstrual patterns (25). Fourth, although major confounders were adjusted for in the multivariate analysis, unmeasured factors such as inflammation, subclinical infections, or genetic hemoglobinopathies may still have influenced hemoglobin outcomes (26)

In addition, while the six-month follow-up period was sufficient to detect hematologic improvement, it may not adequately reflect the long-term sustainability of dietary behaviors or adherence to supplementation once structured supervision is withdrawn (27). The supplementation protocol, although standardized using an intermittent dosing regimen (60 mg twice weekly for 12 weeks), may also be influenced by individual variability in absorption and adherence. Finally, the interventions were delivered with close monitoring by research staff and teachers, potentially creating conditions that may not reflect real-world implementation within routine school health programs. These limitations emphasize the need for caution when interpreting the results and highlight the importance of future research involving biochemical indicators, multi-site sampling, longer follow-up durations, and evaluation of program feasibility under normal, non-supervised conditions.

Recommendations for Future

The results of this study are in strong agreement with previous evidence demonstrating that integrated, multi-component interventions yield greater improvements in hemoglobin levels than single-modality approaches. The present findings showed that the integrated intervention group achieved an increase of 3.5 g/dL substantially higher than the 1.6 g/dL and 2.2 g/dL improvements observed in the diet + education and supplementation + education groups, respectively. These values closely parallel findings from earlier school-based programs in low- and middle-income countries (LMICs), where iron supplementation alone typically produced gains of approximately 1.5–2.0 g/dL, while combined dietary modification and supplementation achieved increases between 2.8 and 3.6 g/dL. Such consistency reinforces the conclusion that addressing anemia requires simultaneous improvements in nutrient intake, absorption, and behavioral adherence.

Moreover, most earlier studies examined supplementation or dietary strategies in isolation, limiting their ability to address the multifactorial nature of adolescent anemia. In contrast, this study implemented three complementary strategies simultaneously—dietary modification, iron supplementation, and structured nutritional education—producing a more comprehensive intervention model. This is particularly relevant given that adolescent anemia arises from a combination of inadequate iron intake, poor absorption, menstrual blood loss, and limited nutritional. By incorporating all three components, the current study provides stronger evidence that multi-dimensional interventions are consistently more effective than single-modality approaches. These comparative insights highlight the necessity for integrated strategies in public health programs aimed at reducing anemia among adolescent girls.

CONCLUSION

This study shows that an integrated approach combining dietary modification, iron supplementation, and nutritional education is the most effective strategy for improving hemoglobin levels among adolescent girls. Although all interventions increased hemoglobin, the integrated model produced the greatest improvement, highlighting the greater comparative effectiveness of addressing both biological and behavioral factors simultaneously. These findings support the adoption of multi-component, school-based anemia programs within national health policies, particularly in high-burden settings. Further research using biochemical markers, longer follow-up periods, and broader populations is needed to strengthen evidence for large-scale implementation.

AUTHOR CONTRIBUTION STATEMENT

Dewi Erna Marisa led the study conception, design, data analysis, and manuscript drafting as the corresponding author. Erida Fadila supported study design refinement, data collection, and interpretation. Ahmad Syaripudin handled instrument development, data acquisition, and statistical analysis. Hasril Desiathul Hamdani conducted the literature review, contributed to the theoretical framework, and assisted with manuscript editing. Lily Wahyuni and Tantri Maulani Putri contributed to the introduction and discussion, provided critical revisions, and assisted in final manuscript preparation. All authors reviewed and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest related to the publication of this manuscript.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

The authors affirm that no generative AI or AI-assisted technologies were used to generate, analyze, or interpret the research data reported in this manuscript. AI tools were used solely to assist with language refinement and formatting, and all content was carefully reviewed, verified, and approved by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the manuscript.

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