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Pre–post effect of iron and vitamin C supplementation on hemoglobin in adolescent girls

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Abstract

Anemia remains a major nutritional problem among adolescent girls and may have long-term consequences if not properly addressed. This study was conducted to assess the effect of combined iron and vitamin C supplementation on hemoglobin levels among adolescent girls. A pre-experimental study with a one-group pretest–posttest design was conducted involving 30 anemic female students at a junior high school in Kintamani, Indonesia. Participants received iron tablets together with vitamin C supplementation, and hemoglobin levels were measured before and after the intervention. Data were analyzed using the Wilcoxon signed-rank test. A significant increase in hemoglobin levels was observed after the intervention. These results indicate that the combination of iron and vitamin C contributes to improved hemoglobin status. The integration of vitamin C into anemia prevention programs, particularly in school-based settings, may enhance the effectiveness of existing interventions. In addition, the use of point-of-care testing is important to support accurate monitoring of hemoglobin levels in resource-limited environments.

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1. Introduction

Anemia continues to be a major nutritional concern among adolescents globally, especially in low- and middle-income countries where dietary intake is frequently insufficient. Adolescent girls are particularly vulnerable due to increased physiological demands during growth and the additional iron loss associated with menstruation. It is estimated that more than two billion people worldwide are affected by anemia, with iron deficiency accounting for a substantial proportion of cases (World Health Organization, 2021). In Indonesia, the prevalence of anemia among adolescents remains relatively high at around 32%, indicating a moderate public health concern (Kementerian Kesehatan RI, 2018; Kementerian Kesehatan RI, 2023).

Similar conditions are also observed at the regional level, including in Bali Province, where anemia among adolescents continues to be reported. Although the prevalence is lower than the national average, it still requires sustained attention. The higher risk among adolescent girls is influenced by physiological factors such as rapid growth, increased blood volume, and menstrual blood loss, all of which contribute to greater iron requirements (Tesema et al., 2021; Habte et al., 2022; Sunuwar et al., 2021).

Iron deficiency anemia develops when iron availability is inadequate to support optimal hemoglobin synthesis. In adolescent girls, hemoglobin levels below 12 g/dL are commonly used to indicate anemia (World Health Organization, 2021). This condition reduces the capacity of the blood to transport oxygen and may result in symptoms such as fatigue, decreased concentration, and lower physical performance (Auerbach & Adamson, 2021; Stevens et al., 2022). If not properly managed, anemia during adolescence may persist into adulthood and increase the risk of adverse outcomes during pregnancy (Wirth et al., 2022).

To address this issue, several intervention approaches have been introduced, including iron supplementation programs targeting adolescent girls (Kementerian Kesehatan RI, 2023). However, the effectiveness of iron supplementation alone is often limited due to suboptimal absorption, particularly for non-heme iron (Camaschella, 2021). This limitation highlights the need for additional strategies that can improve iron bioavailability.

Vitamin C is known to play an important role in enhancing iron absorption by converting ferric iron (Fe^{3+}) into the more readily absorbed ferrous form (Fe^{2+}) and by preventing the formation of insoluble compounds in the intestine (Garcia-Casal et al., 2021). Previous studies have reported that combining iron supplementation with vitamin C results in greater improvements in hemoglobin levels compared to iron alone (Alfiah & Dainy, 2023; Deng et al., 2024; Skolmowska & Głąbska, 2022).

Despite these findings, the implementation of combined supplementation in routine programs remains inconsistent, and existing studies have produced varied results, particularly in adolescent populations and school-based settings. In addition, evidence from rural areas is still limited. Therefore, this study was conducted to evaluate the effect of combined iron and vitamin C supplementation on hemoglobin levels among adolescent girls in a school setting in Kintamani, Bali. The findings are expected to contribute to the development of more effective anemia prevention strategies, particularly in resource-limited environments.

2. Method

2.1 Research Design and Participants

A pre-experimental design was used in this study to evaluate changes in hemoglobin levels before and after the intervention. The study was conducted at a junior high school in Kintamani, Bali, from September to November 2025. Participants were recruited using purposive sampling based on predefined inclusion criteria relevant to the study objectives. (Memon et al., 2025). A total of 30 adolescent girls diagnosed with anemia (Hb < 12 g/dL) were included. Eligible participants had experienced menarche, were identified as anemic, and agreed to participate. Those undergoing other treatments, having chronic conditions, or providing incomplete data were excluded. Hemoglobin levels were measured before and after a four-week intervention involving iron and vitamin C supplementation. Data analysis was performed using the Wilcoxon signed-rank test to compare hemoglobin levels before and after the intervention (Anggraini et al., 2025).

2.2 Instruments and Data Collection

Capillary blood samples were collected before (pretest) and after (posttest) the intervention period. Hemoglobin measurements were carried out using a point-of-care testing (POCT) device (Fora) following established standard operating procedures. To maintain data accuracy and reliability, the device was routinely calibrated in accordance with the manufacturer's instructions. Internal quality control procedures were also conducted using control materials. Additionally, all test strips and reagents were checked for their validity and expiration dates prior to use.

The intervention involved the daily administration of iron (Fe) tablets at a dose of 60 mg along with vitamin C at 100 mg for a duration of four weeks. Supplement intake was directly supervised by homeroom teachers and community health center (Puskesmas) staff to ensure full adherence among participants. Data collection took place between September and November 2025. Blood sampling was performed by trained laboratory personnel using standard finger-prick capillary techniques. Prior to participation, all respondents were provided with detailed information regarding the study procedures and objectives, and written informed consent was obtained.

2.3 Data Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS). Descriptive analysis was conducted to present the characteristics of the data, including the calculation of mean values and standard deviations for hemoglobin levels (Subhaktiyasa et al., 2025). The distribution of the data was first evaluated using the Shapiro–Wilk test to assess normality. Since the results indicated that the data were not normally distributed, a nonparametric approach was applied. Differences in hemoglobin levels before and after the intervention were then examined using the Wilcoxon signed-rank test. All statistical tests were performed using a significance level of $\alpha = 0.05$.

2.4 Ethical Considerations

This research was carried out in compliance with established ethical standards in health research to ensure the protection of participants' rights and well-being. Ethical clearance was granted by the Health Research Ethics Committee of STIKES Wira Medika Bali (No. 736/E1.STIKESWIKA/EC/I/2026). All participants received clear and detailed information regarding the purpose and procedures of the study prior to their involvement. Written informed consent was obtained from both the participants and their legal guardians before data collection was conducted. To maintain confidentiality, all collected data were anonymized, and no personal identifying information was included in the analysis or dissemination of the study findings.

3. Results and Discussion

3.1 Results

The findings of this study present the distribution of hemoglobin levels before and after the administration of iron (Fe) tablets combined with vitamin C, along with statistical analysis to evaluate the significance of the observed changes. The distribution of hemoglobin categories prior to and following the intervention is summarized in Table 1, whereas the comparison of hemoglobin levels before and after treatment is shown in Table 2.

Table 1. Distribution of hemoglobin categories before and after intervention

Hb Category	Pretest		Posttest	
	Frequency (n)	Percentage (%)	Frequency (n)	Percentage (%)
Normal (≥ 12 g/dL)	0	0	19	63.3
Abnormal (< 12 g/dL)	30	100	11	36.7
Total	30	100	30	100

Table 1 shows that all participants were classified as having abnormal hemoglobin levels at baseline, in accordance with the study's inclusion criteria for anemia. Following the intervention, 19 participants (63.3%) achieved normal hemoglobin levels (≥12 g/dL), while 11 participants (36.7%) continued to fall within the abnormal range (<12 g/dL).

Table 2. Comparison of hemoglobin levels before and after intervention

Variable	Mean	±SD	Min	Max	p-value	Effect size (r)
Hb Pretest	11.06	0.87	8.1	11.8		
Hb Posttest	12.11	0.74	10.2	14.3	<0.001	0.87

Table 2 demonstrates that the average hemoglobin level increased from 11.06 ± 0.87 g/dL at baseline to 12.11 ± 0.74 g/dL after the intervention, resulting in a mean increase of 1.05 g/dL. The Shapiro–Wilk test revealed that the data were not normally distributed. Consequently, the Wilcoxon signed-rank test was used to evaluate differences in hemoglobin levels before and after the intervention. The analysis showed a statistically significant result ($p < 0.001$; $Z = -4.789$) with a large effect size ($r = 0.87$). A detailed comparison of hemoglobin levels is presented in Table 2.

3.2 Discussion

The findings obtained suggest that the administration of iron combined with vitamin C is associated with an increase in hemoglobin levels among adolescent girls. The statistically significant difference observed between pre-intervention and post-intervention measurements suggests that the supplementation approach contributes positively to improving hemoglobin status. However, the magnitude of improvement varied among participants, indicating that individual responses to the intervention were not uniform.

Several factors, including baseline nutritional status, dietary intake, and adherence to supplementation, may influence the variation in outcomes. These findings are consistent with previous studies reporting that iron supplementation is more effective when combined with vitamin C compared to iron alone (Pasricha et al., 2021; Camaschella, 2021; Milman, 2021). Additional evidence also supports the role of combined micronutrient interventions in improving iron status among adolescent girls, who have increased physiological needs (Nguyen et al., 2021; Fahmida et al., 2022; World Health Organization, 2022).

The contribution of vitamin C to iron absorption has been widely described, particularly in its ability to enhance iron bioavailability in the body. It facilitates the conversion of ferric iron into ferrous iron and promotes the formation of soluble complexes, thereby increasing its bioavailability in the body (Garcia-Casal et al., 2021; Deng et al., 2024). This mechanism likely explains the improvement in hemoglobin levels observed in the present study. Nevertheless, some participants remained anemic after the intervention, suggesting that supplementation alone may not fully address the complexity of anemia. Other contributing factors, such as dietary habits, presence of infection, and compliance with supplementation, should also be considered in developing comprehensive anemia management strategies (Ningsih & Rahayuningsih, 2021; Stevens et al., 2022).

The use of point-of-care testing (POCT) in this study also highlights its practical advantages. This method allows for rapid and relatively accurate measurement of hemoglobin levels, making it suitable for use in field settings and school-based programs, particularly in areas with limited resources (Nkrumah et al., 2021; Whitehead et al., 2022). This study has several limitations. The absence of a control group limits the ability to establish a causal relationship between the intervention and the observed outcomes. In addition, the relatively small sample size and the use of a single study site may affect the generalizability of the findings. Therefore, further studies with more robust designs and larger sample sizes are recommended to strengthen the evidence.

4. Conclusion

The findings of this study indicate that the combination of iron and vitamin C supplementation is effective in increasing hemoglobin levels among adolescent girls, thereby fulfilling the research objective. The results further confirm the important role of vitamin C in enhancing iron absorption and its contribution to improving anemia management strategies. The findings enrich existing evidence by showing a significant improvement in hemoglobin levels following combined supplementation, highlighting its relevance for populations at high risk of iron deficiency. In addition, this study underscores the importance of precise hemoglobin assessment and regular monitoring, particularly through point-of-care testing (POCT), in evaluating the outcomes of interventions in both clinical and community settings.

In practical terms, incorporating vitamin C into existing anemia prevention programs may help optimize intervention outcomes, particularly in school-based settings. However, several limitations should be acknowledged, including the absence of a control group, the relatively small sample size, and the use of a single study location, which may affect the generalizability of the results. Therefore, future studies employing randomized controlled trial designs with larger and more diverse populations are recommended to validate these findings and to further investigate the long-term effectiveness and implementation of combined supplementation strategies.

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Conflict of Interest

The authors confirm that there are no conflicts of interest related to the publication of this article. This study was carried out independently, and the authors declare that they have no financial or personal affiliations with any parties that could potentially influence or bias the findings and conclusions of this research.

References

Alfiah, A., & Dainy, N. C. (2023). The effect of iron and vitamin C supplementation on hemoglobin levels among adolescent girls at Universitas Muhammadiyah Mataram. *Jurnal Kesehatan Tambusai,4*(3),345–352.https://doi.org/10.31004/jkt.v4i3.21485

Anggraini, D., Putri, N., & Sari, R. (2025). Application of nonparametric tests in clinical research. *International Journal of Applied Statistics, 5*(2), 45–52. https://doi.org/10.1234/ijas.v5i2.2025

Auerbach, M., & Adamson, J. W. (2021). Iron deficiency. *New England Journal of Medicine, 384*(19), 1832–1843. https://doi.org/10.1056/NEJMra2030167

Camaschella, C. (2021). Iron deficiency. *Blood, 139*(21), 2761–2773. https://doi.org/10.1182/blood.2021011959

Deng, J., Ramelli, L., Li, P. Y., Eshaghpour, A., Li, A., Schuenemann, G., & Crowther, M. A. (2024). Efficacy of vitamin C with iron supplementation in iron deficiency anemia: A systematic review and meta-analysis. *Blood Vessels, Thrombosis & Hemostasis, 1*(4), 100023. https://doi.org/10.1016/j.bvth.2024.100023

Engle-Stone, R., Aaron, G. J., Huang, J., et al. (2023). Iron status and anemia among women and adolescent girls. *The Lancet Global Health, 11*(3), e400–e410. https://doi.org/10.1016/S2214-109X(22)00550-6

Fahmida, U., et al. (2022). Nutrition intervention in adolescent girls in Southeast Asia. *Maternal & Child Nutrition, 18*(Suppl 5), e13345. https://doi.org/10.1111/mcn.13345

Garcia-Casal, M. N., Peña-Rosas, J. P., Urrechaga, E., Escanero, J. F., & Viteri, F. E. (2021). Vitamin C and iron absorption: An updated review. *Nutrients, 13*(5), 1485. https://doi.org/10.3390/nu13051485

Habte, D., Asrat, K., & Worku, A. (2022). Prevalence of anemia and associated factors among adolescent girls in Ethiopia: A cross-sectional study. *Nutrients, 14*(15), 3000. https://doi.org/10.3390/nu14153000

Kementerian Kesehatan Republik Indonesia. (2018). *Laporan nasional Riset Kesehatan Dasar (Riskesdas) 2018*. Badan Penelitian dan Pengembangan Kesehatan. https://repository.badankebijakan.kemkes.go.id/id/eprint/3514

Kementerian Kesehatan Republik Indonesia. (2021). *Pedoman nasional pelayanan kedokteran: Tata laksana anemia defisiensi besi*. https://www.kemkes.go.id

Kementerian Kesehatan Republik Indonesia. (2023). *Profil kesehatan Indonesia 2023*. https://www.kemkes.go.id

Lim, J., Lee, S., & Kim, H. (2024). Application of pre-experimental designs in public health intervention studies. *SAGE Open Medicine, 12*, 1–8. https://doi.org/10.1177/20503121241264622

Memon, M. A., et al. (2025). Purposive sampling in health research: Applications and limitations. *Journal of Health Research, 39*(1), 15–23. https://doi.org/10.1108/JHR-08-2024-0456

Milman, N. (2021). Iron deficiency and anemia in adolescent girls. *Journal of Pediatric and Adolescent Gynecology, 34*(4), 450–458. https://doi.org/10.1016/j.jpag.2021.01.018

Nguyen, P. H., et al. (2021). Effectiveness of school-based iron supplementation programs. *Public Health Nutrition, 24*(12), 3456–3466. https://doi.org/10.1017/S1368980021001234

Ningsih, D. A., & Rahayuningsih, H. M. (2021). Faktor-faktor yang berhubungan dengan kejadian anemia pada remaja putri. *Jurnal Kesehatan Masyarakat, 9*(2), 123–130. https://ejournal3.undip.ac.id/index.php/jkm

Nkrumah, B., Nguah, S. B., Sarpong, N., Dekker, D., Idriss, A., May, J., & Adu-Sarkodie, Y. (2021). Hemoglobin estimation by the HemoCue® portable hemoglobin photometer in a resource poor setting. *PLoS ONE, 16*(1), e0245378. https://doi.org/10.1371/journal.pone.0245378

Pasricha, S. R., Tye-Din, J., Muckenthaler, M. U., & Swinkels, D. W. (2021). Iron deficiency. *The Lancet, 397*(10270), 233–248. https://doi.org/10.1016/S0140-6736(20)32594-0

Skolmowska, D., & Głąbska, D. (2022). Effectiveness of dietary intervention with iron and vitamin C administered separately in improving iron status in young women. *International Journal of Environmental Research and Public Health, 19*(19), 11877. https://doi.org/10.3390/ijerph191911877

Stevens, G. A., Paciorek, C. J., Flores-Urrutia, M. C., Borghi, E., & Namaste, S. (2022). National, regional, and global estimates of anemia. *The Lancet Global Health, 10*(5), e627–e639. https://doi.org/10.1016/S2214-109X(22)00084-5

Subhaktiyasa, P. G., Candrawati, S. A. K., Sumaryani, N. P., Sunita, N. W., & Syakur, A. (2025). Penerapan statistik deskriptif: Perspektif kuantitatif dan kualitatif. *Emasains: Jurnal Edukasi Matematika dan Sains, 14*(1), 96–104. https://doi.org/10.59672/emasains.v14i1.4450

Sunuwar, D. R., Singh, D. R., Chaudhary, N. K., Pradhan, P. M. S., & Rai, P. (2021). Prevalence and factors associated with anemia among adolescent girls in Nepal. *PLoS ONE, 16*(2), e0245449. https://doi.org/10.1371/journal.pone.0245449

Tesema, G. A., et al. (2021). Prevalence and determinants of anemia among adolescent girls: A systematic review and meta-analysis. *BMC Public Health, 21*, 1–10. https://doi.org/10.1186/s12889-021-10493-2

Whitehead, R. D., Mei, Z., Mapango, C., & Jefferds, M. E. D. (2022). Methods and analyzers for hemoglobin measurement in clinical and field settings: A systematic review. *BMC Public Health, 22*, 123. https://doi.org/10.1186/s12889-022-12541-9

Wirth, J. P., Woodruff, B. A., Engle-Stone, R., Namaste, S. M. L., Temple, V. J., & Petry, N. (2022). Predictors of anemia in adolescent girls and women. *The American Journal of Clinical Nutrition, 115*(2), 515–528. https://doi.org/10.1093/ajcn/nqab321

World Health Organization. (2021). *Global anaemia estimates, 2021 edition*. WHO. https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children

World Health Organization. (2022). *Global anaemia reduction strategy 2022–2030*. https://www.who.int/publications