ORIGINAL

Effectiveness of Combined Vitamin D and Iron Supplementation on Iron Status in Children with Iron Deficiency Anemia : A Randomized Controlled Trial

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Abstract : Introduction : Vitamin D may reduce inflammation by affecting cytokine production, potentially alleviating chronic anemia. Iron deficiency can impair vitamin D absorption, yet the interaction between these deficiencies remains unclear. This study aimed to evaluate the effectiveness of combined vitamin D and iron supplementation in treating iron deficiency anemia (IDA) in children. Methods : An open-label, randomized controlled trial was conducted at four outpatient clinics and Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. Of 250 assessed patients, 67 met the inclusion criteria. Participants were randomized into two groups : Group A received iron supplementation (3 mg/kg BW/day), while Group B received iron combined with vitamin D (400 IU). Treatment lasted 4 weeks, and iron status was evaluated pre- and post-treatment. Results : No significant changes were observed in complete blood count, serum iron, and ferritin levels after 4 weeks in either group ($p > 0.05$). However, the mean change in serum ferritin levels was significantly greater in the combined group ($p = 0.039$). Conclusion : Both iron alone and iron with vitamin D improved hematological parameters in children with iron deficiency anemia. Combined iron and vitamin D therapy showed a significant ferritin increase, suggesting that adding vitamin D may support better iron storage. J. Med. Invest. 72 : 337-342, August, 2025

Keywords **:** *Anemia, Vitamin D, Iron, Iron-Deficiency, children*

INTRODUCTION

Iron and vitamin D are essential micronutrients for children's optimal growth and development, yet deficiencies in these nutrients are often overlooked (1, 2). Iron deficiency anemia (IDA) is the most prevalent illness and a public health issue worldwide, affecting people of all ages, particularly children aged 6–24 months. The primary cause of IDA in this age group is frequently linked to poor nutritional intake (3, 4). Vitamin D deficiency (VDD) in childhood arises because of an insufficient period of skin exposure to ultraviolet light, or, similar to IDA, because of poor nutritional intake (3). A coexistence of vitamin D and iron deficiencies has been reported, with cross-sectional studies demonstrating correlations between low serum 25-hydroxyvitamin D (s-25(OH)D) levels and reduced serum iron (s-iron), erythrocyte counts, and transferrin saturation (5, 6).

Vitamin D deficiency and anemia have been linked in multiple studies worldwide, with significant associations reported between iron deficiency anemia and vitamin D insufficiency. The presence of vitamin D receptors in the bone marrow and the markedly higher levels of 1,25-dihydroxyvitamin D (1,25-$(OH)_2D$)—the active form of vitamin D—within the bone marrow underscore its critical role in erythropoiesis, the process by which red blood cells (RBCs) are formed (7). Vitamin D regulates hemoglobin synthesis by directly stimulating erythroid precursors, suggesting its substantial role in erythropoiesis (8). Furthermore, vitamin D aids in iron storage and retention and reduces proinflammatory cytokines (9, 10). Consequently, vitamin D deficiency may impair RBC production and function.

Vitamin D can also modulate systemic cytokine production, reducing the inflammatory environment that contributes to chronic anemia. Conversely, iron deficiency may impair vitamin D absorption, much like it reduces the intestinal absorption of fats and vitamin A. Although the exact nature of the relationship between these deficiencies remains unclear, it is crucial to investigate their interplay to develop improved treatment strategies. Therefore, this study aimed to evaluate the effectiveness of combined vitamin D and iron supplementation on iron status in children with iron deficiency anemia, seeking to provide insights into optimizing therapeutic approaches for managing IDA in children.

MATERIALS AND METHODS

Study Design

This open-label, controlled, randomized trial was conducted at four outpatient clinics and Dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia, from April to October 2023. Approved by the Research Ethics Committee of the Faculty of Medicine, Hasanuddin University (approval number 200/UN4.6.4.5.31/PP36/2023, protocol number UH23020108), written informed consent was obtained from the parents or guardians of all participants.

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Participants

Children aged 1-5 years with iron deficiency anemia (IDA) were included. IDA was diagnosed with hemoglobin (Hb) <11 g/dL, MCV <70 fl, MCH <27 pg, and serum ferritin <30 ng/mL. Children who had received previous iron preparations or blood transfusions were excluded. Participants who refused blood tests after 4 weeks were considered dropouts. Children missing more than three consecutive days of medication were excluded, and vitamin D supplementation was stopped after 1 month.

Interventions

Participants were randomly assigned in a 1 : 1 ratio to two groups. Group A, the control group, received iron supplementation at a dose of 3 mg/kg body weight (BW) per day. Group B, the intervention group, received a combination of iron supplementation (3 mg/kg BW per day) and vitamin D supplementation (400 IU per day) for 4 weeks. The iron dosage of 3 mg/kg BW per day was based on clinical guidelines for the treatment of iron deficiency anemia in children, which recommend 3–6 mg/kg/day of elemental iron depending on the child's age and anemia severity (11). Meanwhile, the vitamin D dosage of 400 IU per day aligns with current recommendations for children aged 0–6 months, which suggest a daily intake of 400 IU (10 μg) of cholecalciferol from the first days of life, regardless of feeding method (12).

Both groups were monitored and followed up for 4 weeks. After the treatment period, follow-up tests were performed to assess changes in complete blood count (CBC), serum iron, and serum ferritin levels.

Sample size

The sample size was calculated using the Snedecor and Cochran formula to estimate the number of participants needed to detect a significant difference in iron status between groups. A two-tailed unpaired *t*-test was used, as the study aimed to evaluate whether combined vitamin D and iron supplementation had any significant effect—positive or negative—on children with iron deficiency anemia. Assuming equal group sizes and standard deviations, the formula used was :

$$n = \frac{2\sigma^2 (Z\alpha + Z\beta)^2}{(\mu 1 - \mu 2)^2}$$

$$= \frac{2(0.8)^2 (1.96 \times 1.28)^2}{(0.6)^2}$$

$$= 37.3 \text{ rounded off to } 37$$

Thus, the total sample size required was approximately 37 subjects, which was rounded down to 37. However, due to practical limitations, the minimum acceptable sample size was set to 30. The random allocation sequence was generated using a randomized table, and the allocation was implemented following this table, which was created by the main researcher and other research panels.

Biochemical Analysis

Children's venous blood samples (2 mL) were collected from the median cubital vein by medical professionals. The samples were collected twice : once before and once after 4 weeks of therapy. The complete blood count was performed using K2 EDTA tubes. After centrifugation, serum specimens were obtained for analysis. Serum iron and ferritin levels were measured spectrophotometrically using the ARCHITECT C4100I autoanalyzer. Hemoglobin (Hb), RBC, hematocrit (Hct), MCV, MCH, reticulocytes, and other parameters were analyzed using a hematology autoanalyzer.

Statistical Analysis

The main results of this study were changes in laboratory parameters, including hemoglobin, RBC, MCV, MCH, reticulocytes, serum iron, and serum ferritin, before and after the administration of iron and vitamin D supplementation. The Kolmogorov-Smirnov test was used to assess normal distribution. For normally distributed data, the t-test was applied, while for non-normally distributed data, the Mann-Whitney test was used. Paired data comparisons within each group before and after treatment were performed using the Pearson test. The results were entered into a research form, grouped, and analyzed using SPSS version 26.0 software. A statistically significant result was defined as $P < 0.05$.

RESULTS

Out of 250 children examined for eligibility, 67 fulfilled the inclusion criteria, resulting in a participation rate of 26.8%. The eligible participants were randomly assigned into two different groups with two different therapies. After 4 weeks of therapy, 57 (85%) participant s completed the study, while 10 (15%) participants considered dropping out. (Figure 1)

The baseline characteristics of the subjects were comparable between the Iron Supplementation and Iron + Vitamin D Supplementation groups. There were no significant differences in sex distribution, age, weight, or height between the groups ($p > 0.05$ for all comparisons), ensuring a balanced starting point for analysis. (Table 1)

Within-group analyses revealed significant improvements in several parameters after therapy (Table 2). Both groups demonstrated significant increases in hemoglobin levels, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH). Serum iron levels also increased significantly in both groups, with p-values of 0.018 for the Iron Supplementation group and 0.035 for the Iron + Vitamin D group. Notably, serum ferritin levels showed a statistically significant increase only in the Iron + Vitamin D group ($p = 0.039$). While the Iron-only group demonstrated a larger absolute increase in ferritin, the greater variability in responses may explain the borderline statistical result ($p = 0.052$).

After 4 weeks of therapy, there were no statistically significant differences between the Iron Supplementation group and the Iron + Vitamin D Supplementation group in hemoglobin levels, red blood cell (RBC) count, MCV, MCH, reticulocyte percentage, platelet count, serum iron, or serum ferritin levels ($p > 0.05$ for all parameters), indicating that the addition of vitamin D did not lead to significantly different outcomes compared to iron alone (Table 3).

DISCUSSION

This is the first study in Indonesia to examine the effect of vitamin D supplementation on iron-deficiency anemia in children. Iron and vitamin D are essential micronutrients for the optimal development of young children, and deficiencies in these nutrients remain significant health issues in developing countries like Indonesia. Such deficiencies can adversely affect children's physical growth, immunity, infection risk, and neurocognitive development (13, 14).

The results of this study indicate that four weeks of daily iron supplementation combined with vitamin D supplementation did not result in significant differences compared to iron supplementation alone in terms of complete blood count, serum

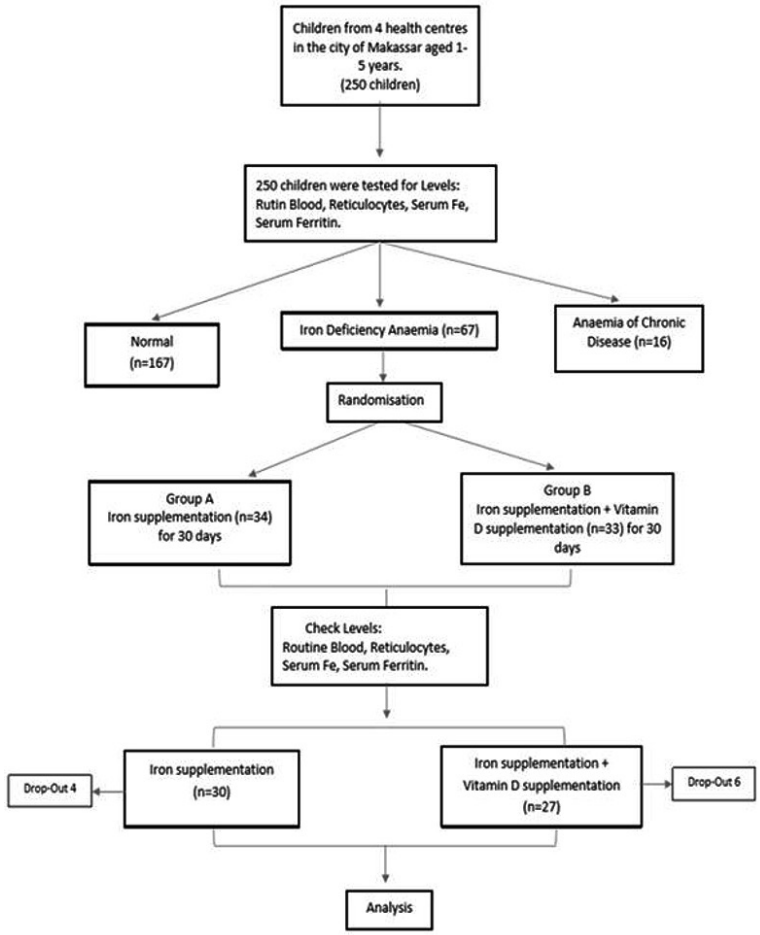

Figure 1. Flowchart of Participant Recruitment, Randomization, and Analysis in the Study

Table 1. Baseline characteristics of the subjects

	Iron supplementation (n = 30)	Iron + Vitamin D supplementation (n = 27)	P value
Sex			
Male	22 (73.3%)	19 (70.4%)	0.804*
Female	8 (26.7%)	8 (29.6%)	
Age			
<2 years	24 (80%)	20 (74.1%)	0.594*
≥2 years	6 (20%)	7 (25.9%)	
Weight (kg)			
Median (min-max)	8.72 (6-26)	8.1 (6.1-21)	0.337**
Height (cm)			
Median (min-max)	73.2 (62-110)	71 (61-110)	0.276**

*Uji *Chi-Square* Test
** Mann-Whitney U Test

Table 2. Mean differences in hematological and biochemical parameters within groups before and after therapy

Variables	Iron supplementation (n = 30)	P value	Iron + Vitamin D supplementation (n = 27)	P value
Hemoglobin (gr/dl)				
Baseline	9.66 (1.25)	0.000**	9.04 (1.98)	0.000**
After 4 Weeks	10.95 (1.34)		10.82 (1.67)	
RBC (10^3/ul)				
Baseline	4.76 (0.44)	0.526*	4.86 (0.54)	0.619*
After 4 Weeks	4.70 (0.61)		4.91 (0.65)	
MCV (pq)				
Baseline	66.49 (7.74)	0.001**	62.77 (9.02)	0.000**
After 4 Weeks	71.83 (8.38)		70.31 (10.00)	
MCH (fl)				
Baseline	20.57 (3.64)	0.000**	18.91 (4.35)	0.000**
After 4 Weeks	23.63 (3.70)		22.42 (4.80)	
Reticulocyte (%)				
Baseline	1.49 (0.84)	0.252*	1.26 (0.70)	0.542*
After 4 Weeks	1.32 (0.90)		1.36 (0.67)	
Platelet				
Baseline	475366 (137442)	0.013**	446555 (131408)	0.044**
After 4 Weeks	406433 (124876)		387814 (130301)	
Serum Fe				
Baseline	34.43 (27.80)	0.018**	1.27 (0.70)	0.035**
After 4 Weeks	67.20 (64.82)		1.37 (0.67)	
Serum Ferritin				
Baseline	10.03 (8.87)	0.052*	9.9 (9.39)	0.039**
After 4 Weeks	170 (433)		114 (252.05)	

*Paired T-Test
**Statistically significant (P < 0.05)

Table 3. Comparison of post-treatment hematological and biochemical parameters between the Iron and Iron + Vitamin D groups after 4 weeks of therapy

Variables	Iron supplementation (n = 30)	Iron + Vitamin D supplementation (n = 27)	P value
Hemoglobin (gr/dl)			
Mean (SD)	1.29 (1.79)	1.78 (1.65)	0.210*
RBC (103/ul)			
Mean (SD)	-0.05 (0.49)	0.05 (0.59)	0.428*
MCV (pq)			
Median (min-max)	3.10 (-5-33.20)	7 (-2-28.40)	0.111*
MCH (fl)			
Median (min-max)	2 (-0.10-16)	2.2 (0-11.80)	0.390*
Reticulocyte (%)			
Mean (SD)	-0.17 (0.80)	0.09 (0.95)	0.249*
Platelet			
Mean (SD)	-68.93 (143000)	-58.74 (144169)	0.79*
Serum Fe			
Median (min-max)	13.85 (-102-245)	8.00 (-39-246)	0.538*
Serum Ferritin			
Median (min-max)	19.6 (-11.29-1998.05)	16.60 (-7.50-1190.85)	1.00*

* Mann-Whitney U Test

iron, and serum ferritin levels. These results differ from those of a randomized placebo-controlled trial conducted on Spanish women, which found higher hematological parameter values in the iron plus vitamin D group compared to the iron-only group (15). While these findings suggest a potential benefit of vitamin D supplementation, it's important to note that such studies were conducted in adult populations. Therefore, the effects may not be directly transferable to children, given differences in physiology, baseline nutritional status, and developmental needs.

Several studies, mostly in adults, have reported mixed results on the interaction between vitamin D and iron status. For example, Basutkar *et al*. found that vitamin D treatment did not enhance clinical outcomes such as hemoglobin and ferritin levels in iron-deficient patients (16). Similarly, a systematic review noted no significant effect of varying vitamin D dosages on hemoglobin and ferritin levels, likely due to the high heterogeneity of the included studies. Conversely, some research suggests that vitamin D may play a role in erythropoiesis by stimulating erythroid progenitor cell proliferation and maturation (17, 18). However, evidence from pediatric populations also highlights the relevance of vitamin D in iron metabolism. A recent study involving children with type 1 diabetes mellitus found that those with vitamin D deficiency had significantly lower levels of hemoglobin, serum iron, ferritin, and transferrin, along with elevated inflammatory markers such as IL-6 and CRP, compared to children with sufficient vitamin D levels (19). These findings suggest that vitamin D deficiency may contribute to both absolute and functional iron deficiency in children, possibly through inflammation-mediated mechanisms. The hormone 1,25-dihydroxyvitamin D has been shown to influence erythropoiesis, and its deficiency may hinder this process (10, 20, 21).

Our findings align with the principal outcome of a meta-analysis that found no association between vitamin D therapy and improvements in anemic patients. However, Smith *et al*.'s narrative review highlighted that cholecalciferol supplementation improved anemia by modulating pro- and anti-inflammatory cytokines, reducing hepcidin levels, and enhancing iron availability (17). These discrepancies suggest that the benefits of vitamin D supplementation may depend on various factors, including the presence of inflammation, parathyroid hormone levels, and fibroblast growth factor 23 activity (5, 22).

In this study, a significant improvement in hemoglobin, MCV, and MCH levels was observed following four weeks of iron supplementation. The frequency of subsequent monitoring should depend on the severity of anemia, the underlying cause of iron deficiency, and the clinical impact on the patient. For moderate to severe anemia, reassessment is recommended as early as 2–4 weeks, with hemoglobin expected to increase by 10-20 g/L within that period. Replenishing iron stores may take up to 6 months (23). While serum iron increased significantly, serum ferritin showed only a marginal difference in the iron supplementation group. When combined with vitamin D supplementation, there was no significant improvement in hemoglobin, MCV, MCH, or serum iron levels compared to iron alone. However, serum ferritin levels showed a more consistent and statistically significant increase. This suggests that vitamin D may play a role in optimizing erythropoiesis and enhancing iron replenishment, possibly through its influence on iron metabolism and other regulatory factors.

The strengths of this study include its design as a randomized controlled trial and its assessment of anemia and iron deficiency anemia through key measures of iron status. However, the study had some limitations, including a small sample size and the inability to collect data on participants' dietary habits, which could have provided insight into their iron intake. Additionally, to optimize the effects of vitamin D, a higher dose, such as 1000 IU, may be beneficial. Further research with larger sample sizes and dose optimization is needed to confirm these findings.

This study concludes that both iron alone and combined iron–vitamin D therapy improved serum iron levels and hematological parameters in children with iron deficiency anemia after 4 weeks. The combined therapy did not significantly affect hemoglobin or red cell indices, but it led to a statistically significant increase in serum ferritin, suggesting that vitamin D may help support more consistent improvement in iron storage.

CONFLICTS OF INTEREST

All authors have disclosed no conflicts of interest.

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REGISTRATION

This trial has been registered in ClinicalTrials.gov with registration number NCT06148545. It can be accessed on https://clinicaltrials.gov/study/NCT06148545.

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