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RESEARCH ARTICLES

Multiple Micronutrient Supplementation on Hemoglobin Levels among Second-Trimester Pregnant Women

Suplementasi Multiple Mikronutrien terhadap Kadar Hemoglobin pada Ibu Hamil Trimester Kedua

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Abstract

Anemia during pregnancy remains a major maternal health concern, particularly in the second-trimester when physiological hemodilution may reduce hemoglobin levels. Iron-folic acid (IFA) supplementation is the standard intervention for preventing and treating maternal anemia; however, multiple micronutrient supplementation (MMS) has been proposed as a potentially more comprehensive approach due to its broader nutrient content. This study aimed to compare the effectiveness of MMS and IFA in improving hemoglobin levels among second-trimester pregnant women. A quasi-experimental pretest-posttest non-equivalent control group design was conducted among 34 pregnant women. Participants were selected using purposive sampling and assigned to either the MMS group (n=17) or the IFA group (n=17). Hemoglobin levels were measured before and after the intervention using the EasyTouch GCHb point-of-care device. Data were analyzed using paired t-tests and independent t-test/Welch's t-test with a significance level of 0.05. Baseline hemoglobin levels were similar in both groups (9.82 g/dL). Following the intervention, mean hemoglobin levels increased to 10.47 g/dL in the IFA group and 11.38 g/dL in the MMS group. The mean increase in hemoglobin was significantly greater in the MMS group (1.56±0.59 g/dL) compared with the IFA group (0.65±0.05 g/dL), with a between-group difference of 0.91 g/dL (95% CI 0.61–1.21; $p < 0.001$; Hedges' $g = 2.14$). MMS was significantly more effective than IFA in increasing hemoglobin levels among second-trimester pregnant women. These findings suggest that MMS may provide greater benefits in addressing maternal anemia during pregnancy.

Keywords: hemoglobin, multiple micronutrient supplementation, pregnancy

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Abstrak

Anemia selama kehamilan masih menjadi masalah kesehatan maternal yang utama, terutama pada trimester kedua ketika hemodilusi fisiologis dapat menurunkan kadar hemoglobin. Suplementasi zat besi dan asam folat (IFA) merupakan intervensi standar untuk mencegah dan mengatasi anemia maternal. Namun, suplementasi multi mikronutrien (MMS) dianggap sebagai pendekatan yang berpotensi lebih komprehensif karena mengandung berbagai zat gizi esensial yang lebih luas. Penelitian ini bertujuan untuk membandingkan efektivitas MMS dan IFA dalam meningkatkan kadar hemoglobin pada ibu hamil trimester kedua. Penelitian quasi-eksperimental dengan desain pretest-posttest non-equivalent control group dilakukan pada 34 ibu hamil. Partisipan dipilih menggunakan teknik purposive sampling dan dialokasikan ke dalam kelompok MMS (n=17) atau kelompok IFA (n=17). Kadar hemoglobin diukur sebelum dan sesudah intervensi menggunakan alat point-of-care EasyTouch GCHb. Data dianalisis menggunakan uji paired t-test dan independent t-test/Welch's t-test dengan tingkat signifikansi 0,05. Kadar hemoglobin awal pada kedua kelompok serupa (9,82 g/dL). Setelah intervensi, rata-rata kadar hemoglobin meningkat menjadi 10,47 g/dL pada kelompok IFA dan 11,38 g/dL pada kelompok MMS. Rata-rata peningkatan kadar hemoglobin secara signifikan lebih besar pada kelompok MMS (1,56±0,59 g/dL) dibandingkan kelompok IFA (0,65±0,05 g/dL), dengan perbedaan antarkelompok sebesar 0,91 g/dL (95% CI 0,61–1,21; p < 0,001; Hedges' g = 2,14). MMS secara signifikan lebih efektif dibandingkan IFA dalam meningkatkan kadar hemoglobin pada ibu hamil trimester kedua. Temuan ini menunjukkan bahwa MMS berpotensi memberikan manfaat yang lebih besar dalam mengatasi anemia maternal selama kehamilan.

Kata Kunci: *hemoglobin, kehamilan, suplementasi multi mikronutrien*

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Highlight:

- Multiple Micronutrient Supplementation (MMS) was significantly more effective inincreasing hemoglobin levels among second-trimester pregnant women (mean increase of 1.56 g/dL) compared to standard Iron-Folic Acid (IFA) supplementation (mean increase of 0.65 g/dL)
- Following the four-week intervention, the mean hemoglobin level in the MMS group successfully reached the normal threshold (11.38 g/dL), whereas the IFA group remained in the mild anemia category (10.47 g/dL)
- MMS demonstrated a very large clinical benefit (Hedges' g = 2.14), with an additional hemoglobin increase of 0.91 g/dL compared to the IFA control group.

INTRODUCTION

Maternal anemia remains a major public health concern worldwide. In 2021, the prevalence of anemia during pregnancy reached 35.5% globally and 41.8% in Southeast Asia. In Indonesia, although the prevalence has declined from 48.9% in 2018 to 27.7% in recent national surveys, approximately one in four pregnant women still experiences anemia (Abdilahi et al., 2024). This condition is associated with various adverse maternal and neonatal outcomes, including preterm birth, low birth weight, small-for-gestational-age infants, and increased perinatal mortality (UNICEF, 2024). Therefore, reducing maternal anemia remains an important strategy for improving maternal and child health and achieving the Sustainable Development Goals (SDGs) 2030.

For many years, iron and folic acid (IFA) supplementation has been the primary intervention for preventing and treating anemia during pregnancy. However, anemia is often caused not only by iron deficiency but also by deficiencies of other essential micronutrients such as vitamins A and B12, zinc, copper, and iodine, which play crucial roles in erythropoiesis, oxygen transport, and cellular metabolism. Consequently, IFA supplementation alone may not adequately address the multifactorial causes of anemia, particularly during pregnancy when nutritional demands increase substantially. To overcome this limitation, multiple micronutrient supplementation (MMS), containing 15–18 essential vitamins and minerals, has been introduced as a more comprehensive nutritional intervention.

Growing evidence suggests that MMS is superior to conventional iron-folic acid (IFA) supplementation. Previous studies have demonstrated that MMS is more effective in improving maternal hemoglobin concentration and reducing the risks of low birth weight, small-for-gestational-age births, and perinatal mortality (Keats et al., 2019). Recent evidence and policy recommendations from the World Health Organization (WHO) further support the use of MMS as a comprehensive antenatal nutritional intervention because it provides a broader range of essential micronutrients than IFA alone and may contribute to improved maternal and birth outcomes (Firoz et al., 2025). Similar findings have been reported in both international and Indonesian studies, showing improved hematological outcomes among pregnant women receiving MMS (Gomes et al., 2022). These findings have encouraged the Indonesian government to gradually incorporate MMS into antenatal nutrition programs as a strategy to address multiple micronutrient deficiencies during pregnancy (Lalusu et al., 2023).

Despite the growing evidence supporting MMS during pregnancy, several important research gaps remain (Ambarwati et al., 2023). Most previous studies have primarily focused on neonatal outcomes, maternal iron status, or pregnancy complications such as gestational hypertension rather than directly comparing the effectiveness of MMS and iron-folic acid (IFA) supplementation on hemoglobin improvement among second-trimester pregnant women (Zgliczynska and Kosinska-kaczynska, 2021). Existing studies have also reported inconsistent findings regarding the magnitude of hemoglobin improvement following MMS supplementation (Meiyanti, 2020). While some studies have demonstrated significantly greater increases in hemoglobin levels among women receiving MMS compared with IFA, others have reported limited or non-significant differences, suggesting that the effectiveness of MMS may be influenced by factors such as supplementation duration, adherence, baseline nutritional status, and population characteristics (Ambarwati et al., 2023). Furthermore, evidence on the comparative effectiveness of MMS and IFA in community-based antenatal care settings remains limited (Kissell et al., 2025). Most studies have been conducted in hospitals, research centers, or large-scale supplementation programs, whereas community midwifery practices represent the primary point of maternal healthcare services for many pregnant women in Indonesia (Kissell et al., 2025). Therefore, further research is needed to evaluate whether MMS provides greater benefits than IFA in improving hemoglobin levels among second-trimester pregnant women receiving routine antenatal care in community settings (Retnaningsih et al., 2025).

The second-trimester is a particularly important period for intervention because physiological plasma volume expansion leads to hemodilution and a natural decline in hemoglobin concentration (Agarwal and Rets, 2021). Although this process is considered normal, inadequate micronutrient intake may exacerbate hemoglobin reduction and increase the risk of moderate-to-severe anemia, preterm birth, and low birth weight (Wibowo and Irwinda, 2015). Consequently, the second-trimester is regarded as a critical and responsive window for nutritional interventions aimed at improving maternal hemoglobin levels. Previous studies have demonstrated the benefits of micronutrient supplementation during pregnancy. A systematic review and meta-analysis by Keats et al. (2019) reported that multiple micronutrient supplementation (MMS) was associated with improved maternal nutritional outcomes and better birth outcomes compared with iron-folic acid (IFA) supplementation alone. Similarly, (Gomes et al., 2022) found that pregnant women receiving MMS experienced greater improvements in hematological indicators, including hemoglobin levels. In Indonesia, (Lalusu et al., 2023) also reported that MMS contributed to improved maternal nutritional status and reduced the risk of anemia during pregnancy. These findings suggest that MMS may provide additional benefits beyond standard IFA supplementation, particularly during the second-trimester when maternal micronutrient requirements increase and hemoglobin levels are more susceptible to decline.

A preliminary assessment at TPMB Eny Islamiati, Bululawang found that the facility routinely provides antenatal care services and has implemented MMS distribution among pregnant women. This setting offers an appropriate community-based context for evaluating the hematological effects of MMS under routine antenatal care conditions. However, comparative evidence between MMS and standard IFA supplementation in primary maternal healthcare settings is still scarce.

Therefore, the novelty of this study lies in its specific focus on evaluating the effectiveness of MMS in increasing hemoglobin levels among second-trimester pregnant women in a community-based midwifery practice while directly comparing its effects with standard IFA supplementation. This study aims to determine whether MMS provides greater improvement in hemoglobin concentration than IFA among second-trimester pregnant women at TPMB Eny Islamiati. The findings are expected to strengthen the scientific evidence supporting antenatal nutritional interventions, guide maternal anemia prevention programs, and contribute to the achievement of maternal and child health targets within the SDGs 2030.

METHODS

This study employed a quantitative approach using a quasi-experimental design with a pretest–posttest non-equivalent control group. The design was chosen to evaluate the effect of Multiple Micronutrient Supplementation (MMS) on hemoglobin (Hb) levels by comparing within-group and between-group changes without random allocation. The study was conducted from January to March 2026 at a community-based private midwifery practice (Tempat Praktik Mandiri Bidan/TPMB) in Bululawang District, Malang Regency, Indonesia, which provides routine antenatal care services and has implemented MMS distribution.

The study population comprised all pregnant women attending antenatal care at the study site who met the eligibility criteria during the study period, totaling 34 participants. The minimum sample size was calculated using a paired-sample formula for pretest–posttest designs with a 95% confidence level ($Z_{1-\alpha/2} = 1.96$), 80% statistical power ($Z_{1-\beta} = 0.84$), a standard deviation of differences of 1.0, and an expected mean difference of 0.5 g/dL, yielding a minimum sample size of 32 participants. To account for a potential 10% dropout rate, the required sample size was increased to 34 participants. Since the total eligible population consisted of 34 pregnant women, all eligible participants were included using a total sampling technique. The participants were equally allocated into two groups: MMS (n = 17) and IFA (n = 17). The inclusion criteria were pregnant women in the second-trimester (13–28 weeks of gestation), willingness to participate by providing written informed consent, and hemoglobin levels classified according to WHO criteria for anemia (Hb <11.0 g/dL). Exclusion criteria included chronic diseases, pregnancy complications, consumption of supplements outside the study protocol, and incomplete participation during the study period.

The independent variable was the type of supplementation (MMS or IFA), while the dependent variable was hemoglobin level measured in g/dL on a ratio scale. MMS was administered as one tablet daily containing multiple essential micronutrients, including iron, zinc, vitamins A and B complex, folic acid, and vitamin C, whereas the control group received one tablet of standard IFA daily. The intervention lasted for four weeks, during which adherence was monitored weekly through observation sheets and participant reports.

Hemoglobin levels were measured twice, before and after the intervention, using a point-of-care EasyTouch GCHb device (glucose, cholesterol, hemoglobin). The device was calibrated prior to use, and capillary blood samples were collected aseptically using sterile lancets. Measurements were recorded in standardized observation forms. Data collection relied on primary data obtained directly from participants, including hemoglobin measurements, adherence monitoring, and baseline characteristics such as age, parity, education, and occupation. Weekly monitoring was conducted either through direct visits or remote communication to ensure compliance and to record any mild side effects.

Data analysis was conducted using statistical software. Univariate analysis was used to describe respondent characteristics and the distribution of hemoglobin levels. Bivariate analysis was performed using a paired samples t-test to evaluate differences in hemoglobin levels before and after the intervention within each group because the data were normally distributed and measured on a ratio scale. To compare changes in hemoglobin levels between the MMS and IFA groups, an independent samples t-test was initially performed. Homogeneity of variance was assessed using Levene's test. Because the assumption of equal variances was not met, Welch's t-test was applied for the final between-group comparison. Statistical significance was set at $\alpha = 0.05$ with a 95% confidence interval.

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Institute of Science and Health Technology, RS dr. Soepraoen Malang, with ethical clearance number No. KEPK-EC/507/II/2026. All research procedures were carried out in accordance with established principles of biomedical ethics. This study was classified as minimal risk, as MMS and IFA are routinely used in antenatal care. Any potential mild side effects, such as gastrointestinal discomfort, were monitored throughout the study period, and appropriate follow-up or referral was provided when necessary.

RESULTS AND DISCUSSIONS

Subject characteristics

A total of 34 second-trimester pregnant women were included in the analysis, with equal allocation to the IFA group (n=17) and the MMS group (n=17). The majority of participants were aged 25–29 years (50.0%), multigravida (52.9%), had at least secondary education (55.8% combining senior high school and higher education), and worked as laborers or farmers (50.0%). These characteristics indicate a relatively homogeneous reproductive-age population with moderate educational attainment and predominantly informal occupational backgrounds, reflecting typical community-based antenatal care users in semi-rural Indonesia. Such profiles are relevant because maternal age, parity, and socioeconomic factors may influence nutritional status, adherence to supplementation, and hematological response.

Table 1. Characteristics of research subjects (n=34)

Characteristics	Frequency (n)	Percentage (%)
Age (years)		
20-24	6	17.7
25-29	17	50.0
30-34	10	29.4
≥35	1	2.9
Parity		
Primigravida	9	26.5
Multigravida	18	52.9
Grand Multigravida	7	20.6
Education		
Elementary School/equivalent	6	17.7
Junior High School/equivalent	9	26.5
High School/equivalent	13	38.2
Diploma/Bachelor's Degree	6	17.6
Occupation		
Housewife	12	35.3
Employee	5	14.7
Laborer/Farmer	17	50.0

Source: Primary data, 2026

Hemoglobin levels before and after the intervention in the control and intervention groups

Table 2 presents the descriptive changes in hemoglobin levels before and after the intervention in the control (iron–folic acid, IFA) and intervention (multiple micronutrient supplementation, MMS) groups. At baseline, the mean hemoglobin levels were comparable between the two groups, with both groups having an average hemoglobin concentration of 9.82 g/dL. Following the intervention, hemoglobin levels increased in both groups. The control group receiving IFA experienced an increase from 9.82 g/dL to 10.47 g/dL, corresponding to an absolute increase of 0.65 g/dL. Meanwhile, the intervention group receiving MMS showed a larger improvement, with hemoglobin levels rising from 9.82 g/dL to 11.38 g/dL, resulting in an absolute increase of 1.56 g/dL. These descriptive findings indicate that although both supplementation regimens were associated with improved hemoglobin levels, the magnitude of increase was greater in the MMS group compared with the IFA group. Table 2 is intended to illustrate the before–after hemoglobin values and the absolute change within each group, while statistical analyses are presented separately.

Table 2. Hemoglobin levels before and after the intervention in the control and intervention groups

Group	Hemoglobin Before (Mean ± SD)	Hemoglobin After (Mean ± SD)	Δ Hemoglobin (After–Before)
Control (IFA)	9.82 ± 0.43	10.47 ± 0.44	+0.65 g/dL
Intervention (MMS)	9.82 ± 0.41	11.38 ± 0.59	+1.56 g/dL

Source: Primary data, 2026

Based on Welch's t-test (Tabel 3), a statistically significant difference was observed in hemoglobin improvement between the MMS and IFA groups ($t = -6.398$; $df = 16.247$; $p < 0.001$). The increase in hemoglobin levels was 0.912 g/dL greater in the MMS group than in the IFA group (95% CI: 0.610–1.213). The effect size was very large (Hedges' $g = 2.14$), indicating a substantial advantage of MMS over standard iron-folic acid supplementation.

Table 3. Differences in hemoglobin changes between the MMS and IFA Groups

Variable	t	df	Mean Difference	Std. Error Difference	95% CI	p-value	Hedges' g
Hb Difference*	-6.398	16.247	-0.912	0.143	-1.213 s.d. -0.610	< 0.001	2.14

Note: Welch's t-test, Differences in mean hemoglobin level changes between the intervention and control groups were analyzed using an independent samples t-test (Welch's correction applied when variances were unequal). Statistical significance was determined at $p < 0.05$

The results showed that the average pre-intervention hemoglobin levels in both the control and MMS groups were 9.82 g/dL. The similarity in baseline hemoglobin values indicates that both groups had similar baseline conditions, so changes occurring after the intervention can be attributed to the treatment provided (Gomes et al., 2022). According to the WHO, a hemoglobin level of less than 11 g/dL in pregnant women is categorized as anemia and is associated with an increased risk of maternal and neonatal complications (Tuncalp et al., 2020).

After the intervention, hemoglobin levels in the control group increased from 9.82 ± 0.43 g/dL to 10.47 ± 0.44 g/dL, with an average increase of 0.65 g/dL. This increase indicates that iron and folic acid supplementation remains effective in improving anemia during pregnancy (Keats et al., 2019). Iron is a major component of hemoglobin, which plays a role in oxygen transport to body tissues. Therefore, iron supplementation can increase red blood cell production and hemoglobin concentration. Folic acid is also necessary for DNA synthesis and red blood cell formation, contributing to the improvement of anemia in pregnant women (Bailey et al., 2015).

In the intervention group, hemoglobin levels increased from 9.82 ± 0.41 g/dL to 11.38 ± 0.59 g/dL, with an average increase of 1.56 g/dL. This significant increase indicates that MMS is more effective than iron and folic acid supplementation alone in increasing hemoglobin levels in pregnant women (Gomes et al., 2022). These results align with a meta-analysis by Keats et al. (2019), which reported that MMS provided greater benefits to maternal micronutrient and hematological status than IFA. MMS contains a combination of various essential vitamins and minerals, such as vitamin A, vitamin B complex, vitamin C, vitamin D, zinc, selenium, and iodine, which synergistically support erythropoiesis.

The vitamin C contained in MMS plays a role in increasing the absorption of non-heme iron by reducing ferric iron to ferrous iron, which is more easily absorbed by the intestine (Stevens et al., 2022). Vitamin B6, vitamin B12, and folate function as cofactors in erythrocyte formation and hemoglobin synthesis, thus helping accelerate the improvement of anemia (Bailey et al., 2015). Vitamin A is known to increase the mobilization of iron reserves from the liver to the blood circulation, thereby increasing iron availability for hemoglobin formation (Gernand et al., 2016). The combination of these micronutrients makes MMS more effective than single supplementation with iron and folic acid.

The statistical test results showed a *p-value* < 0.001, indicating a significant difference between the MMS group and the control group. This value indicates that the increase in hemoglobin in the MMS group was not due to chance but rather a real effect of the intervention (Gomes et al., 2022). The mean difference of -0.912 indicates that the hemoglobin increase in the MMS group was approximately 0.91 g/dL higher than in the control group. A 1 g/dL increase in hemoglobin during pregnancy has been reported to contribute to a reduced risk of persistent anemia and anemia-related obstetric complications (Stevens et al., 2022).

Hedges' g value of 2.14 indicates a very large effect size. According to effect size interpretation, a value above 0.80 is categorized as a large effect, so a value of 2.14 reflects very strong intervention effectiveness. This large effect size indicates that MMS has substantial clinical benefits in increasing hemoglobin levels in anemic pregnant women.

The final hemoglobin level in the MMS group reached 11.38 g/dL, exceeding the normal hemoglobin threshold for pregnancy according to the WHO. In contrast, the average hemoglobin level in the control group remained at 10.47 g/dL, indicating that some subjects were still in the mild anemia category (Tuncalp et al., 2020). These findings suggest that MMS is more effective at enabling pregnant women to achieve normal hemoglobin levels normal hemoglobin levels during pregnancy (Keats et al., 2019). Improving hemoglobin status is crucial because anemia during pregnancy is associated with an increased risk of preterm delivery, low birth weight, postpartum hemorrhage, and maternal and neonatal mortality (Stevens et al., 2022).

Overall, the results of this study indicate that MMS is more effective than IFA in increasing hemoglobin levels in anemic pregnant women (Gomes et al., 2022). These findings support WHO recommendations encouraging the use of MMS in populations at high risk of micronutrient deficiencies (Tuncalp et al., 2020). Therefore, MMS has the potential to serve as an effective nutritional strategy for preventing and managing maternal anemia during pregnancy.

CONCLUSIONS

Multiple Micronutrient Supplementation (MMS) have been shown to be more effective than Iron-Folic Acid (IFA) in increasing hemoglobin levels in anemic pregnant women. The average increase in hemoglobin levels in the MMS group was 1.56 g/dL, higher than the IFA group, which only experienced an increase of 0.65 g/dL. Statistical analysis revealed a significant difference between the two groups with a very large effect size (Hedges' g = 2.14). These findings indicate that MMS is more effective in improving the hemoglobin status of anemic pregnant women than IFA supplementation. Therefore, MMS can be considered as an alternative or complement to supplementation programs during pregnancy to support the prevention and management of anemia and improve maternal and fetal health. The results of this study provide a scientific basis for the development of maternal health policies and programs, particularly in efforts to address anemia in pregnant women. Further research with larger sample sizes and broader coverage is needed to confirm the effectiveness of MMS across various populations of pregnant women.

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