Journal of Multidisciplinary Healthcare

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

The Potential Impact of Dietary Fiber Supplementation on Hemoglobin and Reticulocyte Hemoglobin Equivalent (RET-He) Levels in Pregnant Women with Anemia Receiving Oral Iron Therapy in Indonesia

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Purpose: Anemia during pregnancy can lead to physical and cognitive impairments, fatigue, and postpartum depression. Dietary fiber, as a prebiotic, supports gut health by producing short-chain fatty acids, which enhance immunity and aid iron absorption. This study investigates the impact of fiber supplementation on hemoglobin and reticulocyte hemoglobin equivalent (RET-He) levels in anemic pregnant women receiving oral iron therapy.

Patients and Methods: This study used a quasi-experimental design with a control group. The subjects were anemic pregnant women between 14 and 32 weeks of gestation. Fifteen control subjects received iron tablets and skim milk (placebo), while 25 intervention subjects received iron tablets and a fiber supplement containing 7.2 grams of dietary fiber. Hemoglobin and RET-He levels were measured before and after the intervention with a Sysmex hematology analyzer. The differences in hemoglobin and RET-He changes were analyzed using the independent sample *T*-test.

Results: After 14 days of intervention, the average hemoglobin increase in the intervention group was 0.772±0.815, compared to 0.167±0.564 in the control group, with a p-value of 0.016. There was a significant decrease in the intervention group (p=0.018) and the control group (p=0.008) with normal RET-He values. The average change in RET-He values for the normal group with intervention was −1.44 ± 0.99 and control was −1.63 ± 1.19 (p=0.715), while for the low group with intervention it was 1.65 ± 3.024 and control was 0.55 ± 2.654 (p=0.402).

Conclusion: This study concludes that fiber supplementation for 14 days in pregnant women with anemia can significantly increase hemoglobin levels compared to the control group There was a decrease in RET-He values after the intervention in the normal group and an increase in the low group, although it was not statistically significant.

Keywords: anemia in pregnancy, fiber, hemoglobin, iron tablets, RET-He

Introduction

Anemia is a condition characterized by insufficient hemoglobin in red blood cells to transport oxygen throughout the body.[1] Approximately 40.1% of pregnant women globally, and 48% in Southeast Asia, experience anemia.[2,3] In Indonesia, the prevalence of anemia in pregnant women increased from 37.1% in 2013 to 48.9% in 2018, according to data from the Basic Health Research in Indonesia.[4] In 2019, WHO reported a 44.2% prevalence of anemia among

Received: 24 September 2024
Accepted: 15 December 2024
Published: 17 January 2025

pregnant women aged 15–49 years.[5] An anemia prevalence of ≥ 40% in a population is still classified as a severe public health issue.[3]

Nutritional deficiencies, primarily iron deficiency (75% of cases), are a major cause of anemia.[6] Despite the coverage of 90 iron tablet supplementation for pregnant women reaching 84.2% nationally and 95.3% in West Java, the prevalence of anemia among pregnant women remains notably high.[7] In pregnant women, iron deficiency anemia can impair physical performance, cause fatigue, shortness of breath, cognitive decline, behavioral issues, and postpartum depression. It also affects fetal growth, leading to low birth weight, preterm birth, and increased risk of stunting, along with cognitive, socio-emotional, and motor development issues in children.[7–9]

Pregnant women with anemia exhibit notable gut microbiota changes, particularly in the third trimester, including decreased Firmicutes and increased *Bacteroides* compared to healthy controls.[10] Healthy pregnant women show increased beta diversity and decreased alpha phylogenetic diversity in their microbiota compared to non-pregnant states.[11] Hormonal changes, environment, and diet significantly impact microbiota variations.[12] These findings suggest a link between gut microbiota, pregnancy, and anemia, though causal relationships are not yet clear.

Only about 15% of ingested iron is absorbed in the duodenum; the rest reaches the colon. Oral iron supplementation can disrupt gut microbiota, decreasing commensal bacteria and increasing pathogenic strains like *Bacteroides* and *Enterobacteriaceae*, while reducing *Bifidobacterium* and *Lactobacillus*. Iron that accumulates in the colon can lead to reactive oxygen species (ROS) formation, causing oxidative stress and damaging intestinal epithelial cells.[13,14]

Fiber plays a crucial role in digestive health and iron absorption. However, fiber intake among pregnant women in Indonesia remains low, averaging around 15 g/day.[15] Soluble fiber, which dissolves in water and acts as a prebiotic, supports gut microbiota health and improves its composition.[16–18] Soluble fibers such as inulin, galacto-oligosaccharides, and fructo-oligosaccharides significantly boost Bifidobacteria with low daily doses (5–8 g).[19] Studies on rats given inulin and oligofructose have demonstrated that inulin enhances iron absorption by increasing the levels of divalent metal transporter 1 (DMT1) in the cecum, which facilitates the transfer of iron in its ferrous form, and by boosting duodenal cytochrome B (Dcyt B) in the colon, an enzyme that catalyzes the reduction of ferric iron (Fe^{3+}) to its absorbable ferrous state. Both DMT1 and Dcyt B are crucial for efficient iron uptake.[20,21] Therefore, this study includes additional fiber supplementation to help meet daily fiber requirements and potentially support improved iron absorption.

Research on fiber supplementation in pregnant women with anemia is limited, and existing studies mainly focus on prebiotic fibers that aid iron absorption. This study aims to assess whether fiber supplementation can improve dysbiosis in these women, who are also receiving oral iron therapy. Specifically, it evaluates the impact of fiber supplementation on iron absorption by measuring hemoglobin levels and reticulocyte hemoglobin equivalent (RET-He). Hemoglobin is a protein in red blood cells that reflects iron levels in circulation.[8] Meanwhile, RET-He indicates iron availability for erythropoiesis and serves as an early marker of changes in bone marrow iron levels.[8,22]

Materials and Methods

Study Design and Period

This quasi-experimental study with a pretest-posttest control group was conducted from April to July 2024 at Rancamanyar and Bihbul health centers in Bandung Regency, Indonesia. Blood tests were performed at Hasan Sadikin Central General Hospital's clinical pathology laboratory.

Population of the Study and Inclusion Criteria

The study included pregnant women with anemia from Bihbul and Rancamanyar health centers in Bandung Regency. Eligibility criteria were 14–32 weeks of gestation, Hb < 11 g/dL, mild to moderate anemia (Hb: 7–10.9 g/dL), upper arm circumference > 23.5 cm, leukocytes < 15,000/μL, and normal blood pressure (systolic < 129 mmHg, diastolic < 80 mmHg). Exclusions were made for chronic diseases, comorbidities, acute or chronic diarrhea, recent probiotic use, or irregular iron tablet intake.

Sample Size Determination and Sampling Method

The sample size was calculated using a paired numerical comparative formula, aiming for at least 26 subjects per group to account for potential dropouts. Pregnant women with anemia who met the inclusion criteria on that day were given an equal opportunity to receive a package containing both the test materials and the placebo. Accidental sampling identified 70 eligible pregnant women with anemia, of whom 53 agreed to participate. These participants were randomly assigned to either the intervention group (27 subjects) or the control group (26 subjects). By the end of the study, 40 subjects remained: 25 in the intervention group and 15 in the control group.

Data Collection Instrument and Techniques

Participant data were collected via self-reported forms, interviews, vital signs, physical exams, and anthropometric measurements (weight, height, upper arm circumference). Hemoglobin and RET-He levels were measured using a Sysmex hematology analyzer, with blood samples collected by health center lab staff before and after the intervention.

Participants were divided into two groups: the intervention group received 30 iron tablets (ferrous fumarate with 60 mg elemental iron, 400 mcg folic acid each) and 15 packs of fiber supplement drinks produced by *Kino Biotech Corp*. (37.5 g/serving, with 7.2 g dietary fiber including beta-glucan, inulin, FOS, and GOS), while the control group received the same iron tablets and 15 packs of milk powder produced by *Milko Beverage Industry Corp*. (9 g/serving, 100% cow's milk). Supplements were taken daily for 14 days: iron tablets twice daily, in the morning and at night, and drink powder once daily during the afternoon snack time. Adherence was monitored with checklists, and food intake data were collected using food record forms filled out for three randomly selected days each week, including one weekend day, and analyzed with NutriSurvey2007®. Forms and any remaining supplements were collected on day 15.

Measurement

Hemoglobin and Reticulocyte Hemoglobin Equivalent (RET-He) Levels

Hemoglobin and RET-He levels were measured from a complete blood count using a Sysmex Hematology Analyzer. Hemoglobin was assessed via the Sodium Lauryl Sulfate-Hemoglobin method, and RET-He by Fluorescent Flow Cytometry. Peripheral venous blood samples were drawn with a 3 cc syringe, placed in EDTA tubes, kept at 4–6°C, and sent to the clinical pathology lab at Hasan Sadikin Central General Hospital, Bandung, Indonesia.

Anthropometric Measurements

Anthropometric measurements included weight, height, and mid-upper arm circumference (MUAC). Weight was measured with a GEA digital scale, height with a GEA stadiometer, and MUAC with a non-stretchable tape on the relaxed arm (left for right-handed and right for left-handed), midway between the olecranon and acromion. Subjects with MUAC $\geq$ 23.5 cm were considered well-nourished, while those with MUAC < 23.5 cm were excluded as undernourished.[23]

Subjects Dietary Patterns

Daily food intake was recorded using a food record form for three non-consecutive days within a week, including one weekend day. Participants were guided on how to complete the food record, including details on meal types, preparation methods, and household measurements, with assistance from local health center nutritionists. The data were processed using NutriSurvey2007® software and compared against the recommended dietary allowances from the Indonesian Ministry of Health.

Data Analysis

Data processing involved reviewing measurements (weight, height, food intake, hemoglobin, RET-He levels) and adherence to iron tablets and fiber supplements to ensure data quality and consistency. Data were then coded and analyzed using SPSS version 25.0 for Windows. Univariate analysis described the respondents general characteristics, while bivariate analysis examined relationships between independent and dependent variables. Paired data were used to compare mean differences in hemoglobin and RET-He levels before and after the intervention. The Shapiro–Wilk test assessed data normality ($p > 0.05$ indicated normal distribution). Paired sample *T*-tests were used for normally distributed data, and Wilcoxon tests for non-normal data to compare hemoglobin and RET-He changes. Independent sample *T*-tests and Mann–Whitney tests compared groups for normally and non-normally distributed data, respectively. Significance was determined by $p < 0.05$, with Pearson correlation for normal data and Spearman correlation for non-normal data.

Results

Subjects' Characteristics

During the study, 70 pregnant women with anemia were identified, and 53 agreed to participate. Exclusions included 5 subjects with gestational age > 32 weeks, 1 subject < 14 weeks, 5 with Hb levels > 11 g/dL upon re-testing, and 6 who declined participation. Of the 53 participants, 26 were assigned to the control group and 27 to the intervention group. In the control group 11 subjects dropped out, 5 participants did not respond when contacted, 3 missed follow-up appointments due to lack of transportation and other reasons, and 3 did not consume the package regularly due to nausea after taking it. Meanwhile, in the intervention group, 2 participants dropped out for not responding. Ultimately, 40 subjects completed the study: 15 in the control group and 25 in the intervention group (Figure 1).

Participant characteristics were similar across both groups (Table 1). The average age was 28 years, with weights ranging from 42–83 kg and heights from 145–172 cm. Pre-intervention hemoglobin levels averaged 10.22–10.32 g/dL, with no significant differences between groups. MCV ranged from 62.1–93.2 fL and MCH from 20–33.6 pg, showing no significant variation between groups. Eighty percent had normocytic normochromic anemia, while the remainder had microcytic hypochromic anemia. RET-He levels varied, 22 participants had low RET-He (< 32.1 pg/L) and 18 had normal RET-He (32.1–38.8 pg/L). These were further divided into two groups. No significant differences were found between intervention and control groups for both normal ($p = 0.617$) and low RET-He values ($p = 0.219$).

Daily Dietary Intake Characteristics of Subjects

The analysis showed that the estimated daily fiber intake was significantly higher in the intervention group than in the control group ($p = 0.028$) due to the additional fiber provided. There were no significant differences in the intake of other nutrients between the groups as summarized in Table 2.

Effects of Dietary Fiber Supplementation on Hemoglobin and RET-He Levels

There was a significant difference in hemoglobin levels before and after the intervention in the intervention group ($p = 0.000$). However, in the control group, no significant difference was observed post-intervention ($p = 0.272$) (Table 3).

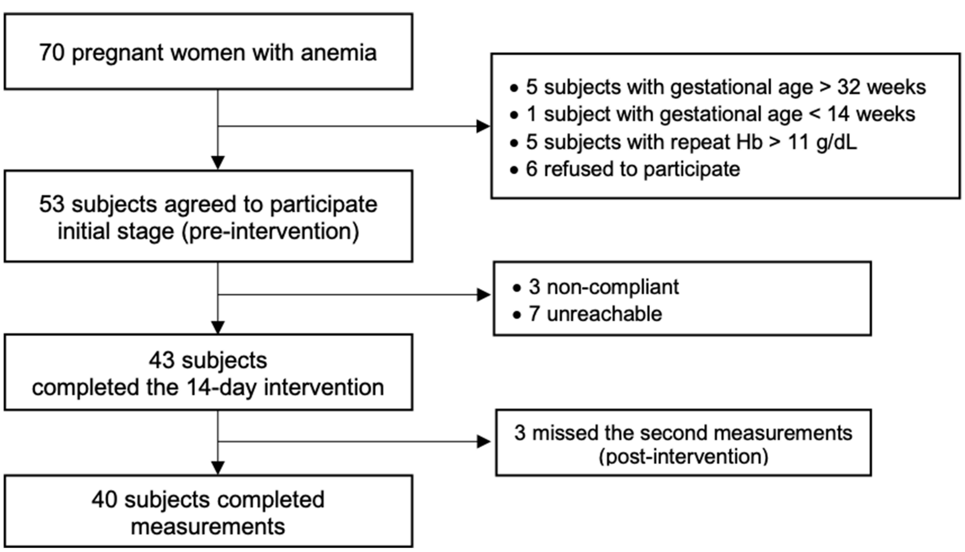

Figure 1 Study enrollment. Fifty-three pregnant women with anemia were initially included and assessed for hemoglobin and Ret-He levels. Ten subjects were excluded, leaving 43 participants who were divided into 2 intervention groups. After 14 days, hemoglobin and Ret-He levels were re-evaluated. Three subjects missed follow-up exams, resulting in final data analysis for 40 participants.

Table 1 Results of the Comparison of Subject Characteristics Between the Two Group

Characteristics	Intervention (n=25)	Control (n=15)	p-value
Gestation/weeks (median/range)[a]	24 (14–30)	19 (14–32)	0.053
Age/years (mean±SD)[b]	28.56±5.53	27.87±6.51	0.721
Parity (median/range)[a]	1 (0–4)	1 (0–4)	0.828
Weight/kg (median/range)[a]	56 (44–83.4)	49 (42–82.6)	0.055
Height/cm (median/range)[a]	154 (145–172)	153 (145.1–159)	0.545
Hemoglobin (g/dL)[b]			
Mean±SD	10.22±0.49	10.32±0.52	0.561
RET-He (pg/L)[a]			
Normal			
Median (Range)	33.2 (32.1–35.1)	32.8 (32.1–35.2)	0.617
Low			
Median (Range)	29.2 (21–31.8)	27.75 (20.6–29.7)	0.219
MCV (fL)[a]			
Median (Range)	88 (65.4–93.2)	84.4 (62.1–92.2)	0.065
MCH (pg)[a]			
Median (Range)	29.8 (20–33.6)	27.8 (20.2–31.5)	0.305
Education[c]			
Elementary School (n/%)	4 (16)	3 (20)	0.493
Junior High School (n/%)	5 (20)	1 (6.67)	
Senior High School (n/%)	13 (52)	7 (46.67)	
Tertiary Education (n/%)	3 (12)	4 (26.67)	
Total (n/%)	25 (100)	15 (100)	
Employment status[c]			
Does not work (n/%)	19 (76)	11 (73.33)	1.000
Work (n/%)*	6 (24)	4 (26.67)	
Total (n/%)	25 (100)	15 (100)	

Notes: [a]mann–Whitney non-parametric test, [b]Independent Sample *T*-Test, [c]Chi-square test. RET-He normal (32.1–38.8 pg), RET-He low (< 32.1 pg).

In both the intervention and control groups with normal RET-He values, there was a significant decrease (p = 0.018 and p = 0.008). However, for those with low RET-He values no significant difference was observed: p-value = 0.087 for the intervention group and p-value = 0.674 for the control group.

Hemoglobin levels increased significantly in the intervention group (0.772±0.815) compared to the control group (0.167±0.564) with p-value = 0.016. There was no significant difference in the change in RET-He values before and after the intervention in each group (Table 4).

Table 2 Comparison of Estimated Daily Nutrient Intake Between Intervention and Control Groups

Nutrition	Intervention (n=25)		Control (n=15)		p-value
	Mean±SD	Median (range)	Mean±SD	Median (range)	
Calorie (kcal)[a]	-	2541.4 (1907.05–3472.90)	-	2459.6 (1761.80–3978.43)	0.086
Carbohydrate (g)[a]	-	334.88 (294.5–482.1)	-	331.9 (284–506.4)	0.645
Protein (g)[b]	98.34±23.05	-	102.2±24.49	-	0.619
Fat (g)[b]	95.94±27.79	-	80.74±34.02	-	0.132
Dietary fiber (g)[b]	24.96±5.75	-	19.92±8.16	-	0.028*
Fe (mg)[a]	-	134.8 (126.2–167.07)	-	138.6 (126.2–151.8)	0.230
Vit. A (mcg)[a]	-	820 (187.40–2886.5)	-	820 (97.80–2867.5)	0.834
Vit. E (mg)[a]	-	4.73 (0.40–25.90)	-	5.6 (0.90–18.20)	0.586
Riboflavin (mg)[b]	1.88±0.83	-	2.14±1.17	-	0.416
Vit. B6 (mg)[a]	-	2.00 (1.30–3.93)	-	2.42 (1.40–4.00)	0.356
Folic acid (mcg)[b]	1143±152.7	-	1269±249.2	-	0.053
Vit. B12 (mcg)[a]	-	4.5 (0.80–15.67)	-	4.42 (1.6–21.5)	0.567
Vit. C (mg)[b]	124.3±64.2	-	138±93.1	-	0.554

Notes: [a]mann–Whitney non-parametric test; [b]Independent sample *T*-test; *Statistically significant at p<0.05.

Table 3 Comparison of Hemoglobin and RET-He Levels Before and After Intervention

Groups	Mean±SD	Median (Range)	T	p-value
Hemoglobin (g/dL)[a]				
Intervention (n=25)				
Before intervention	10.22±0.49	-	−4.738	0.000*
After intervention	10.996±0.99	-		
Control (n=15)				
Before intervention	10.32±0.52	-	−1.144	0.272
After intervention	10.49±0.76	-		
RET-He (pg/L)[b]				
Normal				
Intervention (n=11)				
Before intervention	-	33.2 (32.1–35.1)	-	0.018*
After intervention	-	32.1 (30.5–33.1)		

(*Continued*)

Table 3 (Continued).

Groups	Mean±SD	Median (Range)	T	p-value
Control (n=7)				
Before intervention	-	32.8 (32.1–35.2)	-	0.008*
After intervention	-	31.6 (30.2–33.8)		
Low				
Intervention (n=14)				
Before intervention	-	29.2 (21–31.8)	-	0.087
After intervention	-	31.55 (20.3–34.5)		
Control (n=8)				
Before intervention	-	27.75 (20.6–29.7)	-	0.674
After intervention	-	26.55 (20.3–31.9)		

Notes: [a]Paired sample *T*-test, [b]Non-parametric Wilcoxon test, *Statistically significant at p<0.05. RET-He normal (32.1–38.8 pg), RET-He low (< 32.1 pg).

Table 4 Comparison of Hemoglobin and RET-He Changes Post-Intervention Between Intervention and Control Groups Using Independent Sample *T*-Test

Groups	Mean±SD	95% CI	Mean Difference	T	p-value
Changes in Hemoglobin after Intervention (g/dL)					
Intervention (n=25)	0.772±0.815	(−1.09) – (−1.21)	−0.605	−2.531	0.016*
Control (n=15)	0.167±0.564				
Changes in RET-He after Intervention (pg/L)					
Normal					
Intervention (n=11)	−1.44±0.99	(−0.904) – (1.289)	0.192	0.372	0.715
Control (n=7)	−1.63±1.19				
Low					
Intervention (n=14)	1.65±3.024	(−1.581) – (3.781)	1.100	0.856	0.402
Control (n=8)	0.55±2.654				

Notes: *Statistically significant at p<0.05. RET-He normal (32.1–38.8 pg), RET-He low (< 32.1 pg).

Discussion

This study focused on pregnant women aged 14–32 weeks to maintain uniform hemodynamic conditions. The average age of participants was over 20, with some above 35. Younger women typically have lower iron reserves and may face nutritional challenges due to immaturity. In contrast, older women have reduced iron stores and are more susceptible to anemia due to weaker immunity and age-related health problems.[24,25] Most participants had given birth at least once. Riyani (2020) found a significant link between parity and anemia, while Hidayati (2018) observed that 24 out of 36 anemic women had more than three pregnancies, indicating that higher parity increases the risk of anemia.[26]

Most anemic pregnant women in this study had a high school education. Maternal education influences how food is prepared for the family; those with higher education usually have better knowledge and are more open to dietary

https://doi.org/10.2147/JMDH.S497795

guidance. Working mothers, despite often being more educated, may struggle with providing adequate nutrition due to time constraints and job demands.[27,28]

Anemia is closely related to daily nutrition, with proteins, vitamins A, B6, B12, and folic acid playing direct roles in red blood cell production. Iron is crucial for cellular functions and hemoglobin synthesis, supported by vitamins A, C, and riboflavin in its metabolism. Folate is necessary for DNA and RNA synthesis, with deficiencies affecting nucleus maturation and hemoglobin production. Vitamin B12 is an enzyme cofactor in DNA methylation and vital for red blood cell maturation; deficiencies in vitamin B12 or folate can cause megaloblastic anemia. Vitamins C and E act as antioxidants, protecting red blood cells from oxidative stress.[29]

Adequate carbohydrate and fat intake provide necessary calories, allowing proteins to function properly. In this study, 95% of subjects met their daily calorie needs, while 5% were deficient. However, 62.5% did not meet their carbohydrate intake, likely due to pregnancy-related digestive discomfort leading to reduced portions. Adequate calories ensure sufficient energy for physiological processes, including hemoglobin synthesis, aligning with findings by Abid (2019) and Resmana (2015), which showed a positive link between calorie intake and red blood cell and hemoglobin production. Proper calorie intake spares proteins from being used for energy, supporting healthy red blood cell and hemoglobin formation, while inadequate calories can reduce hemoglobin production and heighten anemia risk. Of the subjects, 92.5% met protein needs, and 85% met fat requirements.[30,31]

Daily fiber needs were unmet in 85% of the subjects through their regular diet. Despite receiving additional fiber from the intervention, only 15% of the subjects achieved their fiber requirements. Adequate fiber intake is crucial for gut health, as it supports a healthy gut microbiota, which in turn enhances digestive health and optimal nutrient absorption.[20,21]

Iron is vital for erythropoiesis, mitochondrial function, DNA replication and repair, electron transport, neurotransmitter synthesis, thyroid function, leukocyte activity, and fatty acid production. During pregnancy, iron needs rise due to fetal and placental growth, increased blood volume, and potential blood loss during childbirth.[24]

Hemoglobin can reflect circulating iron levels. In this study, hemoglobin was used as a parameter to evaluate the effects of fiber supplementation on oral iron therapy in pregnant women with anemia. Results showed a significant increase in hemoglobin levels in the intervention group ($p = 0.000$), while the control group showed a non-significant increase ($p = 0.272$). The effect of fiber supplementation was shown by comparing the increase in hemoglobin levels between the intervention and control groups, with a significant difference ($p = 0.016$) observed in favor of the intervention group. This confirms that fiber supplementation positively impacts hemoglobin levels. The study provided 7.2 g of additional fiber, including prebiotics such as beta-glucan, inulin, FOS, and GOS.

These results align with Jeroense (2019), where 38 young women with iron deficiency showed a significant hemoglobin increase in the group receiving 15 g/day of GOS.[32] Iqbal (2022) found that the highest increase in hemoglobin was achieved with 963 mg/kg GOS combined with 15 ppm Fe_2SO_4, followed by 963 mg/kg inulin with 15 ppm Fe_2SO_4, 963 mg/kg GOS with 30 ppm Fe_2SO_4, and 963 mg/kg inulin with 20 ppm Fe_2SO_4.[33]

Iron deficiency anemia is linked to low MCV and MCH values, indicating small (microcytic) and pale (hypochromic) red blood cells, along with low RET-He levels. RET-He is an early indicator of iron availability for erythropoiesis in the bone marrow and helps detect iron deficiency early. It measures cellular hemoglobin content and can be assessed in real-time through routine lab tests without additional methods. RET-He is more accurate than ferritin and transferrin saturation for detecting iron deficiency in patients with inflammation or chronic conditions. It effectively assesses iron status and therapy response, indicating iron availability for hemoglobin synthesis and serving as a marker for iron deficiency anemia.[34]

In this study, 32 out of 40 subjects had normocytic normochromic anemia, while the remainder had microcytic hypochromic anemia. The fact that 80% of the pregnant women had normocytic normochromic anemia is consistent with Tunkyi's (2017) findings, which showed 65.3% of anemic pregnant women with this morphology.[35] The reasons behind the higher incidence of normocytic normochromic anemia in pregnancy are not fully understood and may relate to pregnancy-related factors. Further research is needed to clarify these causes.

Among the 40 anemic pregnant women, 22 had low RET-He (<32.1 pg/L) and 18 had normal RET-He (32.1–38.8 pg/L), suggesting potential iron deficiency. The decrease in RET-He, despite normal MCV and MCH, indicates that RET-He may serve as an early marker for iron deficiency.[8] Since anemia can have various causes, low hemoglobin can occur with normal RET-He or vice versa. Thus, participants were categorized into normal and deficient RET-He groups.

Analysis revealed significant differences in RET-He levels before and after intervention in the intervention group with normal RET-He ($p = 0.018$), although there was a tendency for RET-He to decrease post-intervention. A similar finding was observed in the control group with normal RET-He ($p = 0.008$). In the intervention group with low RET-He, there was a trend toward increased RET-He post-intervention, but this change was not statistically significant ($p = 0.087$). The control group with low RET-He also showed a trend toward decreased RET-He, with no significant difference ($p = 0.674$).

Fiber supplementation's impact was assessed by comparing RET-He values before and after the intervention. The results showed no significant difference in RET-He changes between the intervention and control groups ($p = 0.715$), and no significant difference for groups with low RET-He ($p = 0.402$). Thus, fiber supplementation did not significantly affect RET-He values.

Dietary fiber serves as a prebiotic, nourishing gut microbes and enhancing gut barrier function, immunity, reducing harmful bacteria, and increasing short-chain fatty acids (SCFAs) production.[19,36] The gut microbiota supports digestion, nutrient metabolism, vitamin synthesis, and immune function.[13] Inulin, oligofructose, and FOS are well-studied prebiotics shown to significantly increase *Bifidobacteria* levels in stool.[19] Inulin has proven effective in enhancing iron absorption. Studies on rats given inulin and oligofructose demonstrated that inulin increases levels of divalent metal transporter 1 (DMT1) in the cecum, responsible for transferring iron in its ferrous form, and boosts duodenal cytochrome B (Dcyt B) in the colon, an enzyme that catalyzes the reduction of ferric iron (Fe^{3+}) to its absorbable ferrous state.[20,21]

Research on fiber supplementation in pregnant women with anemia is limited, and existing studies mainly focus on prebiotic fibers that aid iron absorption. Although evidence suggests that prebiotic fibers can enhance iron bioavailability and improve anemia, research on their impact on RET-He values is sparse, and no comparable studies were found. This study observed a trend of decreased RET-He values post-intervention, except in the group with initially low RET-He. The cause of this decline remains unclear despite iron supplementation. Further research is needed to confirm these findings and their clinical implications.

During the study, no significant side effects were observed during the administration of the test materials. However, some pregnant women reported a change in stool color to dark green, which may also have been influenced by the food intake of the subjects. This study had an insufficient sample size, requiring further research with larger samples to confirm the results. The high dropout rate may be due to inadequate communication between the research team and participants, highlighting the need for improved information dissemination and monitoring in future studies.

Identifying specific causes of anemia, such as thalassemia, in pregnant women is a key limitation of this study. Although RET-He testing is part of the complete blood count panel, its use is restricted to specific hematology analyzers. While RET-He provides insights into hemoglobin content in reticulocytes and reflects iron status, it is not sufficient for diagnosing thalassemia. Accurate diagnosis requires confirmatory tests, such as genetic analysis or hemoglobin electrophoresis. Despite this, RET-He may serve as a preliminary screening tool for potential thalassemia cases. Future research should use more specific diagnostic parameters to better identify anemia's underlying causes and include gut microbiota profiling to evaluate the effects of dietary fiber on gut health.

Conclusion

Based on the data analysis, it can be concluded that fiber supplementation positively impacts hemoglobin levels in pregnant women with anemia undergoing oral iron therapy, though it does not affect RET-He levels. Normocytic normochromic anemia was the most common type, seen in 80% of pregnant women based on red blood cell morphology. Future research should focus on understanding how fiber impacts nutrient absorption, exploring different types and doses of fiber with varying evaluation periods, and assessing potential digestive side effects. Additionally, studying the etiology of anemia in pregnancy using more specific lab tests and investigating undetected minor thalassemia could improve anemia management and help prevent complications during pregnancy and childbirth.

Abbreviations

RET-He, Reticulocyte hemoglobin equivalent; WHO, World Health Organization; ROS, Reactive Oxygen Species; GOS, Galacto-Oligosaccharides; FOS, Fructo-Oligosaccharide; DMT-1, Divalent Metal Transporter-1; DNA, Deoxyribonucleic Acid; RNA, Ribonucleic Acid; MUAC, Mid-Upper Arm Circumference.

Data Sharing Statement

All relevant data and supporting information are included in the paper.

Ethics Approval and Informed Consent

All research methods adhered to the Helsinki Declaration's ethical principles for medical research involving human subjects. Ethical approval was granted by the Health Research Ethics Committee, Faculty of Medicine, Universitas Padjadjaran, Indonesia (Approval No. 33/UN6.KEP/EC/2024). Permissions were also obtained from Rancamanyar Health Center, Bihbul Health Center, the Bandung District Health Office, Agency for National Unity and Politics of Bandung Regency, and Hasan Sadikin General Hospital, Bandung, Indonesia. Informed consent was secured from all participants after they were fully briefed on the study's objectives. Privacy and confidentiality were per the Declaration of Helsinki.

Acknowledgments

We extend our gratitude to Puskesmas Rancamanyar and Puskesmas Bihbul for permitting the research. We also appreciate Hasan Sadikin General Hospital, Bandung, for allowing sample analysis in their clinical pathology laboratory. Additionally, we acknowledge the invaluable contributions of our data collectors, advisors, and study participants.

Author Contributions

All authors significantly contributed to the reported work, encompassing conception, study design, execution, data collection, analysis, and interpretation, or involvement in all these aspects. They participated in drafting, revising, or critically reviewing the article, provided final approval for its publication, agreed on the journal to which the article was submitted, and accept responsibility for all elements of the work.

Funding

This study was self-funded by the main researcher.

Disclosure

The authors declare that they have no competing interests in this work.

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