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The Effects of Vitamin C for Iron Supplementation during Pregnancy with Risk of Anemia: A randomized controlled clinical trial

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ABSTRACT

Objectives: To evaluate the effects of combining vitamin C with iron supplementation on hemoglobin (Hb) and hematocrit (Hct) levels among pregnant women at high risk of anemia.

Materials and Methods: A randomized controlled trial was conducted among singleton pregnant women at 14-28 weeks of gestation, with Hb ≥ 10.5 g/dL, Hct ≥ 32% and ferritin ≥ 30 ng/mL, who were at risk of anemia (> prior 2 pregnancies, age < 18 or > 35 year-old or body mass index < 18 kg/m^2). Women attending Rajavithi Hospital from July 2023 - June 2024 were randomly assigned to receive vitamin C (500 mg) plus iron supplement or iron only. Hb and Hct were measured 2 months after intervention.

Results: Total of 100 enrolled participants, 22 (22%) were excluded for having ferritin levels < 30 ng/mL. The remaining 78 women were randomized, with 38 in the experimental group (vitamin C plus iron) and 40 in the control group (iron only). There were no significant differences in Hb and Hct levels between the two groups, both initially and 2 months after the intervention (Hb: mean difference (MD) -0.11; 95% CI -0.53, 0.31; p = 0.609 and MD -0.24; 95% CI -0.67, 0.32; p = 0.253; respectively) (Hct: MD -0.77; 95% CI -1.96, 0.55; p = 0.266; MD -0.70; 95% CI -1.97, 0.58; p = 0.282). The repeated measure analysis of variance (ANOVA) showed non-significant overall mean differences. No adverse events were reported.

Conclusion: Vitamin C was not essential with iron supplements to improve Hb and Hct levels in pregnant women at risk of anemia.

Keywords: vitamin C, hemoglobin, hematocrit, risk of anemia in pregnancy, iron supplement.

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ประสิทธิภาพของการให้วิตามินซีร่วมกับธาตุเหล็กในหญิงตั้งครรภ์ที่มีความเสี่ยงต่อภาวะโลหิตจาง : การศึกษาทดลองแบบสุ่ม

ลลิตพันธุ์ ศรีมณีศิริ, ลัลธพร พัฒนาวิจารย์

บทคัดย่อ

วัตถุประสงค์: *เพื่อเปรียบเทียบระดับฮีโมโกลบิน (Hemoglobin) และฮีมาโตคริต (Hematocrit) ระหว่างกลุ่มที่ได้รับประทานวิตามินซีร่วมกับธาตุเหล็ก กับกลุ่มที่รับประทานธาตุเหล็กเพียงอย่างเดียว*

วัสดุและวิธีการ: *การแบ่งกลุ่มแบบสุ่มในหญิงตั้งครรภ์เดี่ยวอายุครรภ์ 14-28 สัปดาห์ ที่มีระดับฮีโมโกลบินมากกว่าหรือเท่ากับ 10.5 กรัมต่อเดซิลิตร ฮีมาโตคริตมากกว่า 32 เปอร์เซ็นต์ และระดับเฟอริตินมากกว่าหรือเท่ากับ 30 นาโนกรัมต่อมิลลิลิตร ที่มีความเสี่ยงต่อภาวะซีด (ได้แก่ เคยตั้งครรภ์มากกว่า 2 ครั้ง, อายุน้อยกว่า 18 ปี หรือมากกว่า 35 ปี, ดัชนีมวลกายน้อยกว่า 18 กิโลกรัม ต่อตารางเมตร) ระหว่างเดือนกรกฎาคม 2566 ถึงเดือนมิถุนายน 2567 โดยแบ่งสุ่มเป็นกลุ่มที่ได้รับการเสริมวิตามินซี 500 มิลลิกรัมร่วมกับเหล็ก หรือ การเสริมเหล็กเพียงอย่างเดียว และติดตามการเพิ่มของระดับฮีโมโกลบินและฮีมาโตคริตใน 8 สัปดาห์*

ผลการศึกษา: *จากผู้เข้าร่วมการวิจัยทั้งหมด 100 คน 22 คนถูกคัดออกเนื่องจากระดับเฟอริตินต่ำกว่า 30 คงเหลือ 38 คนในกลุ่มทดลอง (วิตามินซีร่วมกับเหล็ก) 40 คนในกลุ่มควบคุม (เหล็กอย่างเดียว) มี 1รายในกลุ่มนี้ออกจากการศึกษาเนื่องจากทารกเสียชีวิตในครรภ์ ผลพบว่าที่ 8 สัปดาห์ถัดมา ไม่มีความแตกต่างในระดับฮีโมโกลบินและฮีมาโตคริตระหว่าง 2 กลุ่ม (ฮีโมโกลบิน ค่าเฉลี่ยความแตกต่างอยู่ที่ -0.11; 95%CI -0.53, 0.31; p = 0.609 ในกลุ่มทดลองและ -0.24; 95%CI -0.67, 0.32; p = 0.253 ในกลุ่มควบคุม ส่วนฮีมาโตคริตมีค่าเฉลี่ยความแตกต่างอยู่ที่ -0.77; 95%CI -1.96, 0.55; p = 0.266 ในกลุ่มทดลองและ -0.70; 95%CI -1.97, 0.58; p = 0.282 ในกลุ่มควบคุม ไม่มีรายงานภาวะไม่พึงประสงค์จากการรับประทานยา*

สรุป: *การเสริมวิตามินซีร่วมกับเหล็กไม่มีความสำคัญในการเพิ่มระดับฮีโมโกลบินและฮีมาโตคริตในหญิงตั้งครรภ์ที่มีความเสี่ยงต่อภาวะโลหิตจาง*

คำสำคัญ: *วิตามินซี, ฮีโมโกลบิน, ฮีมาโตคริต, สตรีตั้งครรภ์ที่มีความเสี่ยงต่อภาวะซีด, การเสริมเหล็ก*

Introduction

During pregnancy, the blood volume increases by an average of 50% above the non-pregnant level by 34 weeks of gestation[1]. This increase meets the metabolic demands of the enlarged uterus and ensures a sufficient supply of nutrients and elements to support the rapidly growing placenta and fetus. During blood volume expansion, the plasma volume and the erythrocyte mass increase. The average increase in the erythrocyte volume is around 450 mL. However, because the increase in the plasma volume is greater than the increase in the erythrocyte mass, the hemoglobin (Hb) and hematocrit (Hct) levels tend to decrease slightly[2]. On average, the Hb level reaches about 12.5 g/dL, with approximately 5% of pregnant women presenting an Hb level below 11.0 g/dL, particularly in late pregnancy and often due to iron deficiency. Hb is crucial for transporting oxygen in the blood and protecting mothers from complications during childbirth[1,2].

Anemia is a condition characterized by an insufficient number of red blood cells to meet the body's physiological needs. According to the World Health Organization (WHO) guidelines[3], an Hb level below 11.0 g/dL is indicative of anemia in pregnant women. However, according to the American College of Obstetricians and Gynecologists (ACOG) practice bulletin[3], anemia in pregnancy is defined by Hb and Hct levels below 11 g/dL and 33%, respectively, in the first trimester; 10.5 g/dL and 32%, respectively, in the second trimester; and 11 g/dL and 33%, respectively, in the third trimester. Iron deficiency is considered to be the most common cause of anemia. Ferritin has the highest sensitivity and specificity for diagnosing iron-deficiency anemia, with a level below 30 µg/L confirming this condition[4].

In 2019, the global prevalence of anemia among women of reproductive age was 29.9% (95% uncertainly interval [UI] 27.0%, 32.8%), while the prevalence among pregnant women was 36.5% (95% UI 34.0%, 39.1%)[5]. The prevalence of anemia in Thai population in first antenatal care as 6.92%, third trimester was 24.62% and intrapartum period was 4.76%[6]. The prevalence of iron deficiency in early pregnancy Thai population was reported to be 15.2%[7]. The factors associated with anemia include age, gender, residential area, and smoking habits. Additionally, studies have identified correlations with multiple pregnancies (> 2)[5,8,9], teenage pregnancy[8,9], maternal age over 35 years[5], and a low pre-pregnancy body mass index (BMI)[8]. Among pregnant women, iron deficiency anemia is also linked to adverse pregnancy outcomes such as an increased risk of gestational diabetes (15.9%), fetal oxygen abnormalities leading to fetal non-reassuring status (9.4%), preterm delivery (8.2%), a low amniotic fluid level (1.95%), neonatal complications (10.6%), critical care admissions (9.7%), and a low birth weight (4.9%). Moreover, anemia significantly raises the likelihood of requiring blood transfusions during pregnancy[5,8].

The WHO recommends daily oral supplementation of iron and folic acid, with 30-60 mg/day of elemental iron, for pregnant women to prevent maternal anemia[11]. Iron supplements can be administered orally, intramuscularly, or intravenously; each method offers its own benefits and potential side effects. Oral supplementation is generally preferred due to its efficiency, affordability, and safety. Vitamin C enhances iron absorption by helping to reduce ferric iron through the enzyme duodenal cytochrome B (DCYTB), which transfers electrons from intracellular ascorbate. This process underscores the importance of vitamin C in improving iron absorption. Additionally, vitamin C contributes to the formation of low-molecular-weight iron chelates, further increasing iron absorption. It is also linked to erythropoiesis as well as the storage and mobilization of ferritin[12]. Numerous studies have demonstrated that combining vitamin C with iron supplementation significantly increases Hb and Hct levels, making it a safe option for pregnant women and their babies[13]. The objective of this study was to evaluate the effect of combining vitamin C with iron supplementation on Hb and Hct levels in pregnant women at high risk of anemia. Since the goal of this research is to prevent anemia during pregnancy and reduce associated

risks, the study specifically focuses on high-risk groups.

Materials and Methods

This open label randomized controlled clinical trial (CinicalTrials.gov ID. NCT05975125) was conducted between July 2023 and June 2024 at the Department of Obstetrics and Gynecology, Rajavithi Hospital, Bangkok, Thailand. The study protocol was approved by the Rajavithi Hospital ethics committee (number 118/2566, issued on June 28, 2023). All participants were informed about the study and signed a consent form. For participants under 18 years of age, informed consent was obtained from their parent or guardian.

The baseline Hb, Hct, and ferritin serum levels were measured. Then, the participants were randomly divided into two groups: the experimental group received vitamin C (500 mg) in addition to iron supplementation (triferdine, 1 tablet daily, containing iodine 150 µg, ferrous fumarate 185 mg, and folic acid 400 µg). The control group received only iron supplementation (Fig. 1). All women in this study received standard antenatal care and nutritional guidance. The iron supplement and vitamin C were started at a gestational age range between 14 and 28 weeks. All participants were prohibited from taking other medications or supplementary foods at the time of taking the medication. Two months after the trial, the Hb and Hct levels were measured again. Data on dietary habits; the possible side effects of vitamin C; and pregnancy outcomes, including postpartum hemorrhage, blood transfusion, infant weight, gestational age at delivery, and postpartum infant health, were collected. Compliance was monitored by counting the actual number of iron and vitamin C tablets that were returned. All data were analyzed to compare the outcomes between the two groups.

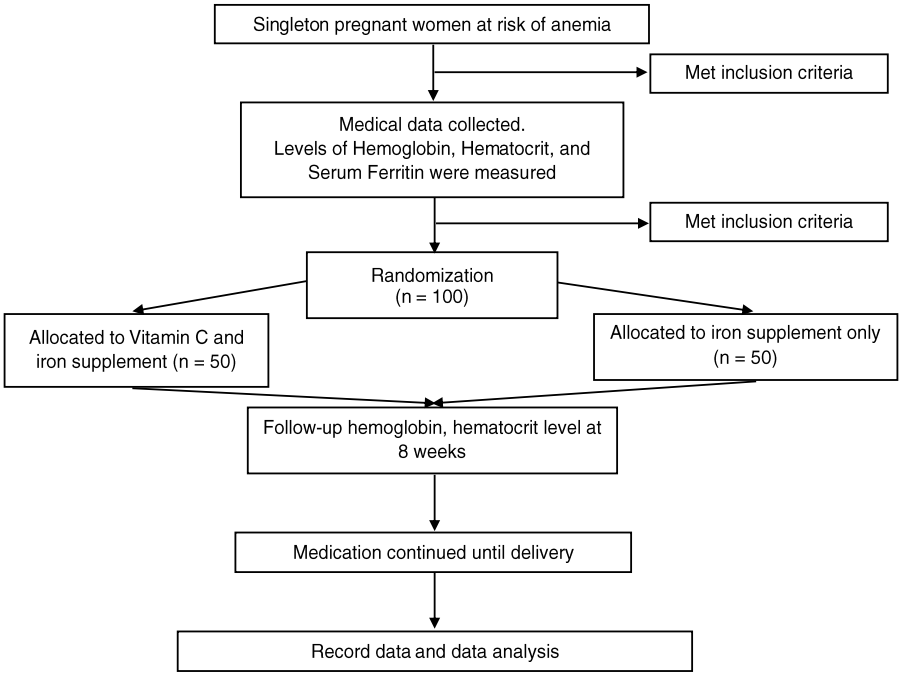

Fig. 1. Consort flow diagram

The primary objective was to compare the ability of iron supplementation, with and without vitamin C, to increase the Hb and Hct levels in pregnant women at high risk of anemia. The secondary objective was to compare pregnancy outcomes between women taking vitamin C plus iron and those

taking iron alone. Pregnant women receiving prenatal care at Rajavithi Hospital and who voluntarily participated in the research project were included. The inclusion criteria were: women with a singleton pregnancy; gestational age between 14 and 28 weeks; and at high risk of anemia due to at least one factor, such as having more than two previous pregnancies, teenage pregnancy, age over 35 years old, or a BMI under 18 kg/m^2. Additionally, the participants needed to be non-anemic, with an Hb level ≥ 10.5 g/dL and an Hct level ≥ 32% for their initial prenatal blood test. The exclusion criteria were: a ferritin level < 30 ng/mL (iron deficiency anemia, based in the ACOG recommendation); received vitamin C supplements during the study; human immunodeficiency virus (HIV) infection; iron deficiency anemia; blood disorders such as thalassemia (either carrier or severe disease), gastrointestinal bleeding, or antepartum hemorrhage; allergy to vitamin C and/or iron; chronic conditions such as kidney disease, liver disease, rheumatism, and abnormal bleeding disorders; a history of iron supplementation within the past 3 months; received blood components within the past 3 months or during the study; and gave birth within the first 2 months of participating in the study. The eligible participants were evenly assigned to the experimental group or the control group at a 1:1 ratio. The randomization process used blocks of 4, meaning that for every set of four participants, an equal number were allocated to each group.

The sample size was calculated based on a previous study[14] by using the following formula.

$$n = \frac{(Z_{\alpha/2}+Z_{\beta})^2 \times (\sigma_1^2+\sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

$$n = \frac{(1.96+0.842)^2 \times (10.89^2+11.43^2)}{(0.83 - 0.84)^2}$$

where n is the sample size, α is 0.05, $Z_{\alpha/2}$ is 1.96, Z_{β} is 0.842, $\sigma 1$ is 0.83, $\sigma 2$ is 0.84, $\mu 1$ is 10.89, and $\mu 2$ is 11.43. The calculation indicated the need for at least 38 participants per group. Considering a 30% drop-out rate, the total sample size was at least 50 participants per group.

SPSS Statistics version 21.0 for Windows (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The continuous variables were presented as the mean ± standard deviation (SD) or the median. The categorical variables were presented as the number and frequency. The participant characteristics were compared the two groups using the chi-square test, Fisher's exact test, or the Mann–Whitney U test. The Hb and Hct levels were analyzed using repeated-measures analysis of variance (ANOVA). A p value of < 0.05 was considered to be statistically significant.

Results

We recruited a total of 100 women with a singleton pregnancy. We excluded 22 participants (22%) with a ferritin serum level < 30 ng/mL. We randomized the remaining 78 women: 38 into the experimental group (vitamin C plus iron) and 40 into the control group (iron-only). All participants completed the 8-week follow-up, except for one in the control group who dropped out due to an unexplained intrauterine fetal death. Thus, at the 8-week follow-up, we measured the Hb and Hct levels, administered surveys, asked about adverse events, and counted the number of pills for 38 participants in the experimental group and 39 in the control group. The participants continued to take the medication until delivery, at which time we recorded the pregnancy outcomes. There was 86.8% compliance in the experimental group and 82.1% compliance in the control group.

Table 1 presents the baseline characteristics for the two groups. The mean age was 30.97 ± 8.88 years in the experimental group and 34.33 ± 7.64 years in the control group (p = 0.169). The mean BMI were 23.16 ± 3.43 kg/m^2 in the experimental group and 24.81 ± 4.19 kg/m^2 in the control group. Most participants had more than two previous pregnancies 51 (66.2%). Regarding the socioeconomic status, 70 participants (90.9%) had an income of < 25,000 baht, 71 (92.2%) had an

education level below a bachelor's degree, and 16 (20.7%) were unemployed. There were no significant differences between the two groups in terms of the baseline characteristics.

Table 1. Baseline characteristics.

Characteristic	Group		p value
	Experimental (n = 38)	Control (n = 39)	
Maternal age (years)			
mean ± standard deviation	30.97 ± 8.88	34.33 ± 7.64	0.169
Body mass index (kg/m^2)			
mean ± standard deviation	23.16 ± 3.43	24.81 ± 4.19	0.141
Serum ferritin levels (ng/ml),			
mean ± standard deviation	90.69 ± 62.61	68.07 ± 36.07	0.055
Parity			0.519
1	8 (21.1)	6 (15.4)	
2	7 (18.4)	5 (12.8)	
> 2	23(60.5)	28(71.6)	
Income			> 0.999
< 25,000 baht	35 (92.1)	35 (89.7)	
≥ 25,000 baht	3 (7.9)	4 (10.3)	
Education			0.431
Less than a bachelor's degree	34 (89.5)	37 (94.9)	
Bachelor's degree and above	4 (10.5)	2 (5.1)	
Occupation			0.081
Unemployed	11(28.9)	5 (12.8)	
Employed	27 (71.1)	34 (87.2)	

P values are from student t-test, Fisher's exact test, or the Mann–Whitney U test.

In this study, data on the participants' dietary habits were collected (Table 2), as these habits can influence iron absorption. Foods such as meat, eggs, dark leafy greens, grains, and beans are rich sources of iron, while vegetables and citrus fruits are high in vitamin C, which enhances iron absorption. Conversely, milk and coffee can inhibit iron absorption. However, no statistically significant differences in dietary intake were observed between the two groups.

Table 3 summarizes the Hb and Hct levels. The baseline Hb and Hct levels did not differ between the groups, with a mean difference of -0.11 (95% confidence interval [CI] -0.53, 0.31, p = 0.609) for Hb and -0.70 (95% CI -1.96, 0.55, p = 0.266) for Hct. At the 8-week follow-up, the mean difference in the Hb and Hct levels were not significantly different between the groups (Hb: -0.24, 95% CI: -0.67, 0.32; p = 0.253; Hct: -0.69, 95% CI -1.97, 0.58, p = 0.282). Table 4 shows the differences of changes in Hb and Hct level between the experimental and control group. The overall mean differences in Hb and Hct levels between the two groups were not significantly different after repeated measure ANOVA analysis (Hb: -0.18, 95% CI -0.55, 0.20, p = 0.350; Hct: -0.70, 95% CI -0.18, 0.42, p = 0.216).

Table 2. Dietary habits of the participants.

Dietary habit	Group		p value
	Experimental (n = 38)	Control (n = 39)	
Meat			0.409
None	0 (0.0)	0 (0.0)	
1–2 days/week	2 (5.3)	6 (15.4)	
3–4 days/week	33 (86.8)	30 (76.9)	
5–6 days/week	3 (7.9)	3 (7.7)	
Every day	0 (0.0)	0 (0.0)	
Eggs			0.050
None	0 (0.0)	0 (0.0)	
1–2 days/week	26 (68.4)	17 (43.6)	
3–4 days/week	12 (31.6)	21 (53.8)	
5–6 days/week	0 (0.0)	1 (2.6)	
Every day	0 (0.0)	0 (0.0)	
Vegetables			0.553
None	4 (10.5)	3 (7.7)	
1–2 days/week	17 (44.7)	12 (30.8)	
3–4 days/week	12 (31.6)	19 (48.7)	
5–6 days/week	3 (7.9)	4 (10.3)	
Every day	2 (5.3)	1 (2.6)	
Citrus fruit			0.508
None	7 (18.4)	8 (20.5)	
1–2 days/week	31 (81.6)	28 (71.8)	
3–4 days/week	0 (0.0)	2 (5.1)	
5–6 days/week	0 (0.0)	1 (2.6)	
Every day	0 (0.0)	0 (0.0)	
Milk			> 0.999
None	1 (2.6)	2 (5.1)	
1–2 days/week	3 (7.9)	4 (10.3)	
3–4 days/week	13 (34.2)	14 (35.9)	
5–6 days/week	18 (47.4)	17 (43.6)	
Every day	3 (7.9)	2 (5.1)	
Dark leafy greens			0.815
None	17 (44.7)	19 (48.8)	
1–2 days/week	19 (50.0)	17 (43.6)	
3–4 days/week	2 (5.3)	3 (7.7)	
5–6 days/week	0 (0.0)	0 (0.0)	
Every day	0 (0.0)	0 (0.0)	

Table 2. Dietary habits of the participants. (Cont.)

Dietary habit	Group		p value
	Experimental (n = 38)	Control (n = 39)	
Grains			0.078
None	8 (21.1)	16 (41.0)	
1–2 days/week	28 (73.7)	19 (48.7)	
3–4 days/week	2 (5.3)	4 (10.3)	
5–6 days/week	0 (0.0)	0 (0.0)	
Every day	0 (0.0)	0 (0.0)	
Beans			0.063
None	34 (89.5)	27 (69.2)	
1–2 days/week	4 (10.5)	11 (28.2)	
3–4 days/week	0 (0.0)	1 (2.6)	
5–6 days/week	0 (0.0)	0 (0.0)	
Every day	0 (0.0)	0 (0.0)	
Coffee			> 0.999
None	37 (97.4)	37 (94.9)	
1–2 days/week	1 (2.6)	1 (2.6)	
3–4 days/week	0 (0.0)	1 (2.6)	
5–6 days/week	0 (0.0)	0 (0.0)	
Every day	0 (0.0)	0 (0.0)	

Data are presented as the number (%).

P values are from Fisher's exact test.

Table 3. The hemoglobin and hematocrit levels.

Variable	Group		mean difference (95% CI)	p value
	Experimental (n = 38)	Control (n = 39)		
Initial hemoglobin, mean ± standard deviation (g/dL)	11.94 ± 0.96	12.05 ± 0.91	-0.11 (-0.53, 0.31)	0.609
Hemoglobin at 8 weeks, mean ± standard deviation (g/dL)	11.48 ± 0.95	11.73 ± 0.89	-0.24 (-0.67, 0.32)	0.253
Initial hematocrit, mean ± standard deviation (%)	36.33 ± 2.70	37.04 ± 2.83	-0.70 (-1.96, 0.55)	0.266
Hematocrit at 8 weeks, mean ± standard deviation (%)	35.10 ± 2.87	35.79 ± 2.74	-0.69 (-1.97, 0.58)	0.282

CI: confidence interval.

P values are from the Mann–Whitney U test.

Table 4. Differences of changes in Hemoglobin and Hematocrit level between the experimental and control group.

Variable	Mean difference of change between experimental and control group (95% CI for repeated-measures analysis of variance)	p value
Hemoglobin at 8 weeks VS baseline	-0.18 (-0.55, 0.20)	0.350
Hematocrit at 8 weeks VS baseline	-0.70 (-0.18, 0.42)	0.216

CI: confidence interval. P values are from the Mann–Whitney U test.

Table 5 summarizes the pregnancy outcomes. The average gestational age at delivery was 38 weeks in the experimental group and 39 weeks in the control group. The mode of delivery included vaginal delivery in 24 cases (63.2%) in the experimental group and 22 cases (56.4%) in the control group, while cesarean section rates were 14 cases (36.8%) in the experimental group and 17 cases (43.6%) in the control group. One case of postpartum hemorrhage was reported in the control group; however, no blood transfusion was required. The estimated blood loss during delivery was 243 mL in the experimental group compared to 278 mL.

Birth weights below 2,500 g were recorded in 8 cases (21.1%) in the experimental group and 3 cases (7.7%) in the control group. Apgar scores below 7 at 1 minute were observed in 33 cases (86.8%) in the experimental group and 36 cases (92.3%) in the control group, while one case (2.6%) in the experimental group had an Apgar score below 7 at 5 minutes. Neonatal intensive care unit (NICU) admissions occurred in 2 cases in each group, accounting for 5.3% in the experimental group and 5.1% in the control group. Overall, there were no significant differences between the two groups in terms of pregnancy outcomes.

Throughout the entire study treatment period, none of the participants reported any side effects from the medications and no adverse outcome occurred.

Table 5. Pregnancy outcomes of the study population between groups.

	Group		p value
	Experimental (n = 38)	**Control (n = 39)**	
Gestational age at birth (weeks)			
mean	38	39	0.922
Route of delivery, n (%)			0.556
Normal labor	24 (63.2)	22 (56.4)	
Cesarean section	14 (36.8)	17 (43.6)	
Postpartum hemorrhage, n (%)			> 0.999
Yes	0 (0.0)	1 (2.6)	
No	38 (100.0)	38 (97.4)	
Estimated blood loss (ml)			0.575
mean ± standard deviation	243 ± 102.30	278 ± 94.52	
Blood transfusion, n (%)	0 (0.0)	0 (0.0)	-
Birth weight, n (%)			0.114
< 2,500 g	8 (21.1)	3 (7.7)	
> 2,500 g	30 (78.9)	36 (92.3)	
mean ± standard deviation	2961.2 ± 340.10	3037.3 ± 393.26	
Apgar score at 1 min, n (%)			0.343
≤ 7	33 (86.8)	36 (92.3)	
> 7	5 (13.2)	3 (7.6)	
Apgar score at 5 min, n (%)			
≤ 7	1 (2.6)	0 (0.0)	
> 7	37 (97.4)	39 (100.0)	
Neonatal intensive care unit admission, n (%)	2 (5.3)	2 (5.1)	> 0.999

P values are from Fisher's exact test or the Mann–Whitney U test.

Discussion

Iron levels can significantly affect pregnancy outcomes, as iron is essential for various physiological processes, particularly the production of hemoglobin, which carries oxygen to tissues, including the placenta and developing fetus[8]. Iron deficiency anemia is a common issue in pregnant women and can lead to adverse pregnancy outcomes. The authors of a previous study reported that the prevalence of anemia increases as pregnancy progresses[8]. The prevalence gradually rises from early pregnancy (2.7%) to mid pregnancy (14.7%), and it peaks in late pregnancy (16.6%). Physiologically, in the third trimester, the velocity of the plasma volume increase slows down, which should elevate the Hb level. However, the rising prevalence of anemia in the third trimester could be attributed to inadequate iron supplementation. Several studies have identified risk factors for anemia during pregnancy[8-10]. These include age > 35 years old, multiple pregnancies, a BMI of < 18.5 kg/m², and a low educational level and income. This study selected participants without iron deficiency anemia, defined as having ferritin levels > 30 ng/mL, based on the ACOG recommendation in the inclusion criteria. However, for follow-up at 8 weeks, the ferritin levels were less useful as a follow-up marker because they were not low at baseline. Anemia during pregnancy has been linked to various pregnancy outcomes. For the prevention of iron deficiency anemia during pregnancy, many recommendations emphasize the importance of incorporating iron supplements into a pregnant woman's routine. This study chose a daily iron dose, as participants were provided with Triferdine, a supplement commonly administered daily during pregnancy and widely used among pregnant women across Thailand. However, it is important to note that iron salts are best absorbed when taken on an empty stomach[15], and studies[16] suggest that administering iron supplements every other day, rather than daily, may enhance absorption. Daily iron supplementation can stimulate the secretion of hepcidin, a hormone that regulates iron metabolism by inhibiting iron absorption in the gut[17]. This physiological response may limit the effectiveness of daily supplementation[16-17]. Therefore, while the use of Triferdine aligned with standard practice in Thailand, this approach may represent a limitation of the study, as it could have influenced the overall efficacy of the intervention.

Vitamin C is known to enhance iron absorption, and previous studies have shown that administering vitamin C alongside iron (in doses of 100–500 mg/day) to pregnant women significantly increases Hb levels without increasing the risk of adverse pregnancy outcomes[14,18,19]. This study selected a dose of 500 mg of vitamin C due to its ease of application (single tablet) and because the absolute dose of vitamin C did not appear to be associated with iron absorption or improvements in iron status[20].

This randomized controlled trial represents the first study designed to assess the effectiveness of oral iron supplementation combined with vitamin C in non-anemic pregnant women who are at risk of developing anemia—referencing the sample group from the aforementioned studies[8–10]. This study focused on prevention and treatment strategies to reduce anemia-related complications for both mothers and infants in this population. Following the intervention, the administration of vitamin C combined with iron supplements did not result in a significant increase in Hb and Hct levels. Moreover, at the 8-week follow-up, Hb and Hct levels slightly decreased in both groups despite continued treatment. These findings were consistent with those of a previous study[8] and may be attributed to physiological plasma volume expansion, inadequate nutrition, or insufficient iron supplementation. In contrast, three prior studies showed that administering vitamin C combined with iron supplements significantly increased the Hb levels[14,18,21]. This difference might be explained by the variations in sample size and population.

This study did not observe a significant reduction in adverse pregnancy outcomes among women who took vitamin C combined with iron supplements compared with the group that only took iron supplements. On the contrary, Hans et al[22] reported that a low birth weight was significantly

reduced in the group of women who received vitamin C compared to those who did not. Additionally, the group receiving vitamin C had a lower rate of hospitalization for several preventable reasons such as anemia in pregnancy, mostly iron deficient anemia (IDA) and respiratory tract infections (RTI) compared with the group that did not receive vitamin C, and the supplementation was deemed safe during pregnancy. During the entire treatment period, there were no serious adverse events. This may be attributed to the fact that we administrated only 500 mg of vitamin C per day, and most of the participants consumed low amounts of vitamin C–rich foods, such as citrus fruits, bell peppers, and tomatoes. Notably, several reports have highlighted the safety of vitamin C during pregnancy[13,23]. The tolerable upper intake level is 2,000 mg per day. Vitamin C is widely regarded as beneficial and non-toxic, with no evidence of it being carcinogen or harmful to the fetus. However, consuming more than 3,000 mg per day may lead to adverse effects, including diarrhea, nausea, vomiting, gastrointestinal discomfort, excessive iron absorption (resulting in iron overload), enamel erosion, kidney stone formation, and reduced vitamin B12 levels.

Dietary intake is a key factor that can influence iron absorption. Whole foods, fortified foods, and supplements serve as the primary sources of iron. Rich sources of heme iron include red meat, poultry, fish, and shellfish. Non-heme iron is also found in foods such as dark leafy greens, nuts, seeds, whole grains, and dried fruits. Consuming vitamin C–rich foods, like citrus fruits, bell peppers, and tomatoes, alongside non-heme iron sources can enhance iron absorption. On the other hand, drinking tea and coffee, which contain phytates, and calcium-rich beverages such as milk, consumed during meals, may reduce non-heme iron absorption[24,25]. Consequently, the findings from other studies on this topic may vary due to differences in daily dietary components, eating habits, dosage of vitamin C, types of vegetables consumed, caffeine intake, or other factors between the groups, which could have affected the interpretation of the results.

This study had several strengths. It minimized selection bias by conducting a randomized controlled trial and including all participants who had prenatal visits and met the inclusion criteria. The study followed all cases until delivery and also documented the dietary habits of each participant. However, the sample size was small, as it focused on preventing anemia in pregnant women without iron deficiency anemia. Further studies with larger sample sizes should be conducted to validate these findings.

Conclusion

This study conducted a randomized controlled trial to assess whether vitamin C and iron supplementation could prevent anemia in pregnant women at risk of developing the condition. Anemia is commonly found in pregnant women and can lead to various complications, as mentioned earlier. Identifying effective preventive measures is crucial, as anemia poses risks not only to maternal health but also to neonatal outcomes. Prevention is always preferable to treatment, as it reduces the burden on healthcare systems and improves overall pregnancy outcomes. However, this study found that administering vitamin C alongside iron did not significantly increase Hb and Hct levels compared to iron supplementation alone. The study acknowledges the limitations, which may have impacted the ability to detect subtle differences between the groups.

Potential conflicts of interest

The author declares no conflicts of interest.

References

1. Soma-Pillay P, Catherine NP, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr 2016;27:89-94.
2. Aguree S. Gernand AD. Plasma volume expansion across healthy pregnancy: a systematic review and meta-analysis of longitudinal studies. BMC Pregnancy Childbirth 2019;19:508.
3. World Health Organization. Haemoglobin concentrations of the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition

Information System. http://apps.who.int/iris/bitstream/10665/85839/3/WHO_NMH_NHD_MNM_11.1_eng.pdf [Accessed 30th August 2024].
4. Anemia in pregnancy. ACOG Practice Bulletin No. 223. Obstet Gynecol 2021;138: e55-e64.
5. World Health Organization. Anaemia in women and children. WHO Global Anaemia Estimates, 2021 Edition. https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children [Accessed 30th August 2024].
6. Palinlat W, Chonlada L, Sakchai P. Prevalence and associated factors of iron-deficient erythropoiesis in Thai pregnant women. Thai J Obstet Gynaecol 2024; 32:391-9.
7. Suphawet L, Budsaba V. Prevalence and associated factors of anemia in different periods of pregnancy. Thai J Obstet Gynaecol 2023;31:56-63.
8. Lin L, Wei Y, Zhu W, Su CWR, Feng H, Yang H. Prevalence, risk factors and associated adverse pregnancy outcomes of anaemia in Chinese pregnant women: a multicentre retrospective study. BMC Pregnancy Childbirth 2018;18:1-18.
9. Abd Rahman R, Idris IB, Isa ZM, Rahman RA, Mahdy ZA. The prevalence and risk factors of iron deficiency anemia among pregnant women in Malaysia: a systematic review. Front Nutr 2022;9.
10. Lestari S, Fujiati II, Keumalasari D, Daulay M, Martina SJ, Syarifah S. The prevalence of anemia in pregnant women and its associated risk factors in North Sumatera, Indonesia. IOP Conference Series: Earth Env Sci 2018;125:012195.
11. World Health Organization. Daily iron and folic acid supplementation during pregnancy. https://www.who.int/tools/elena/interventions/daily-iron-pregnancy [Accessed 30th August 2024].
12. Aznar B, Grandia J, Tejedor MT, Falceto MV. Effect of vitamin C and iron supplementation in pregnant hyperprolific sows. Livestock Sci 2024;280.
13. Panel on dietary antioxidants and related compounds, Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington DC: National Academies Press; 2000.
14. Palupi PD, Khan MS, Karseno KK. Antianemia supplementation combination with vitamin C on hemoglobin levels among pregnant women in primary health care center, Jepara, Indonesia. J Pharma Sci Comm 2021;18: 95-101.
15. Stoffel NU, von Siebenthal HK, Moretti D, Zimmermann MB. Oral iron supplementation in iron-deficient women: How much and how often? Mol Aspects Med 2020;75:100865.
16. Fernandez-Gaxiola AC, De-Regil LM. Intermittent iron supplementation for reducing anaemia and its associated impairments in adolescent and adult menstruating women. Cochrane Database Syst Rev 2019;31:1.
17. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol 2009;122:78-86.
18. Puspita AJ. The effect of iron supplement and vitamin C towards increasing hemoglobin levels in pregnant women with anemia. J Medika Hutama 2021;2: 1060-6.
19. Rumbold A, Ota E, Nagata C, Shahrook S, Crowther CA. Vitamin C supplementation in pregnancy. Cochrane Database Syst Rev 2015;CD004072.
20. Li N, Zhao G, Wu W, Zhang M, Liu W, Chen Q, Wang X. The efficacy and safety of vitamin C for iron supplementation in adult patients with iron deficiency anemia: a randomized clinical trial. JAMA Netw Open 2020;3:e2023644.
21. Murtiningsih M, Lestari L, Fitriani H. The effect of iron and vitamin C supplements on hemoglobin levels of pregnant women with anemia: systematic literature review. J Matern Care Reprod Health 2021;4:31-41.
22. Hans U, Byamukama E. Regular vitamin C supplementation during pregnancy reduces hospitalization: outcomes of a Ugandan rural cohort study. Pan Afr Med J 2010;5:15.
23. Brown B, Wright C. Safety and efficacy of supplements in pregnancy. Nutr Rev 2020;78:813-26.
24. Moustarah F, Daley SF. Dietary iron. https://www.ncbi.nlm.nih.gov/books/NBK540969/ [Accessed 30th August 2024].
25. Harvard Health Publishing. Foods high in iron: incorporating high-iron foods into a healthy diet. https://www.health.harvard.edu/nutrition/foods-high-in-iron-incorporating-high-iron-foods-into-a-healthy-diet [Accessed 30th August 2024].