

ANTIANEMIA SUPPLEMENTATION COMBINATION WITH VITAMIN C ON HEMOGLOBIN LEVELS AMONG PREGNANT WOMEN IN PRIMARY HEALTH CARE CENTER, JEPARA, INDONESIA

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ABSTRACT

Blood volume escalation during pregnancy leads to an increase in iron needs. Pregnant women are prone to maternal anemia that is caused by iron, folic acid, vitamins B₂, B₁₂, A, and C deficiency and may serve as causative factors that aggravate anemia. Concerning the problem, this clinical investigation determined the effect of antianemia supplementation and its combination with vitamin C on hemoglobin levels during pregnancy. This study is a quasi-experimental involving 34 patients who consumed antianemia supplements with or without vitamin C. The treatment group was given a combination of antianemia supplement Fe Fumarate 180 mg-Folic Acid 400 mcg and vitamin C 100 mg. The positive-control group was administered with antianemia supplement Fe Fumarate 180 mg-Folic Acid 400 mcg. After 28 days, blood samples were taken to measure the hemoglobin levels. The hemoglobin levels were found to have a statistically significant difference ($p < 0.05$) between that group receiving a combination of antianemia-vitamin C and the other group administered with antianemia alone.

Keywords: Antianemia supplement; vitamin C; pregnancy; hemoglobin level

INTRODUCTION

The first trimester of gestation is a critical period for the fetus's development. During this period, the structure and organ systems of the fetus are developing. The incidence of miscarriages and congenital disabilities mainly occurs between 3 to 12 fetal weeks (Tsikouras *et al.*, 2020). A high protein diet, oral iron, and folic acid supplementation are recommended for pregnant women to prevent maternal anemia, low birth weight, and preterm birth (Ghattas *et al.*, 2003; Tunçalp *et al.*, 2017). Iron deficiency is the most often encountered nutrient deficiency during pregnancy (Gernand *et al.*, 2016). The leading causes are low oral iron and lack of iron in their diet (Crompton &

Nesheim, 2002; WHO, 2017). Maternal anemia is caused by iron deficiency, while folic acid, deficiency of vitamins B₂, B₁₂, A, and C, and genetically inherited traits such as thalassemia might serve as superimposed causal factors (WHO, 2001). Anemia during pregnancy is identified when hemoglobin (Hb) concentration is < 110 g/L or 10.5 g/L in the second trimester (Munasinghe, 2007; Stevens *et al.*, 2013). The prevention of anemia can be done by consuming iron-rich foods, iron, and folate supplementation combined with vitamin C (Abbaspour *et al.*, 2014; Abu-Ouf & Jan, 2015). The diet of Indonesian women during pregnancy generally contains low iron food.

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The vegetable is known to have low iron quality or non-heme iron (Moustarah & Mohiuddin, 2020; Skolmowska & Głabska, 2019) and requires ascorbic acid to stimulate absorption (Teucher *et al.*, 2004). In terms of its bioavailability, non-heme iron is absorbed much less efficiently than heme iron, which is found in vegetables (Skolmowska & Głabska, 2019). Many studies showed that consuming food supplements such as vitamin C might increase the hemoglobin level during pregnancy (Nemo, 2020; Rimawati *et al.*, 2018; Kadry *et al.*, 2018; Triharini *et al.*, 2019). Nevertheless, a study in Nigeria indicates no correlation between serum iron level and ascorbic acid consumption in the first and second trimester. In contrast, a positive correlation showed in the third trimester. Ascorbic acid plays a vital role in iron intestinal absorption, especially in low serum iron levels (Shu & Ogbodoa, 2005). Iron deficiency anemia can be treated with oral iron supplements. The most commonly prescribed iron consumption is iron sulfate tablet three times daily with a lower dose option. Gastrointestinal disorder is a common side effect of a patient's adherence to iron therapy. Lower dose, iron consumption during meal times or enteric-coated options can avoid this side effect. Still, these strategies interfere with iron absorption and can lead to suboptimal clinical outcomes. Further investigations are needed to co-administrate vitamin C and iron therapy's optimal duration (Fei, 2015).

The benefits of vitamin C consumption to increase the Hb level during pregnancy still have to be investigated. Therefore, this study examined the effect of the combination of Fe supplementation plus vitamin C compared to the antianemia alone on pregnant women's Hb level.

MATERIALS AND METHODS

Study design

The study was quasi-experimental using pre and post control design and conducted over four weeks period. Participants enrolled in this study were recruited from a community health center in Lebak village, Pakis Aji,

Jepara. The subjects were pregnant women in the first and second trimester who volunteered after signing the informed consent and received an antianemia supplement containing Fe Fumarate 180 mg-Folic Acid 400 mcg manufactured by Phapros from Semarang, Indonesia. This study presented the physician prescription pattern of the antianemia supplement in the first and second pregnancy trimester in Pakis Aji primary health care center. Thirty-four participants were divided into two groups. Seventeen patients belonged to the positive-control group receiving antianemia supplement without vitamin C (with mean $\pm$ SD; age 26.41 ± 3.28 years), and the other 17 were placed in the treatment group receiving antianemia supplement combined with vitamin C 100 mg manufactured by Kimia Farma from Jakarta, Indonesia (with mean $\pm$ SD; age 28.18 ± 4.22 years). The supplements were given one tablet per day. Pregnant women with pre-eclampsia were excluded from this study. The Hb level was assayed by collecting the blood samples before administration and after four weeks of treatment using BC-2800 Mindray from China. The sample assessment was done at the Pakis Aji primary health care center laboratory.

Ethical approval

This study has received ethics approval from the Bioethics Committee of Medical Faculty, Sultan Agung Islamic University with the number of 310/V/2019/Komisi Bioetik, and was released on May 22nd, 2019, before the initiation of the study.

Control measurement

The patients were requested to adhere to the dietary plan suggested by the physician. In addition, the physical condition, body weight, blood pressure, and Hb level were recorded, and the outcomes were informed to the patients.

Laboratory examination

Blood samples were collected before the administration of the antianemia supplement and after four weeks of treatment. The Hb levels were analyzed with the

cyanmethemoglobin method (Norsiah, 2015) using Mindray from China. The blood was taken and put into tubes filled with 5 mL of Drabkin's reagent, incubated for 10 minutes, and analyzed with a spectrophotometer at 546 nm wavelengths. The Drabkin's solution's composition is potassium ferricyanide which binds heme (Ferro) to methemoglobin (Ferri), a cyanide ion that changes methemoglobin into cyanmethemoglobin. KH_2PO_4 was added into the solution to regulate the pH between 7.0 to 7.4, and non-ionic detergent was also added to accelerate the lysis of erythrocytes that caused turbidity and interrupted the reading of the spectrophotometer (Gandasoebrata, 2007; McPherson & Pincus, 2011).

Statistical analysis

Data analysis was carried out by univariate and bivariate. First, univariate analysis was performed to determine the socio-demographic frequency distribution of subjects. Simultaneously, the Hb levels

bivariate were represented as mean $\pm$ standard deviation (SD) and were analyzed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). Second, the independent sample T-test was used to assess significant differences between control positive and treatment groups. Finally, a paired student T-test was applied to evaluate differences in group changes before and after the experiment.

RESULTS AND DISCUSSION

According to the univariate descriptive statistical analysis on the socio-demographic characteristic of the participants, most participants' ages (94%) fell in a category within the range of 20 to 35 years (27.29 ± 3.83), and most of the pregnancy (47%) was 20 to 23 weeks. Subject characteristics showed statistically insignificant differences ($p > 0.05$) between the control and treatment groups. The result of the descriptive analysis of participants' socio-demography can be seen in Table 1.

Table 1. Characteristics of subjects receiving an antianemia supplement

Characteristics	Total subjects N (%), (Mean $\pm$ SD)	Positive control group N (%), (Mean $\pm$ SD)	Treatment group N (%), (Mean $\pm$ SD)	p-value
Age (year)	27.29 ± 3.83	26.41 ± 3.28	28.18 ± 4.22	0.183*
Pregnancy (week)	21.56 ± 3.21	19.29 ± 2.57	23.82 ± 1.94	0.00*
16-19	8 (23.5)	2 (11.8)	6 (35.3)	2.00**
20-23	16 (47.1)	6 (35.2)	10 (58.7)	1.00**
24-27	10 (29.4)	9 (53)	1 (6)	6.40**
Body weight (kg)	64.72 ± 2.94	64.88 ± 3.28	64.41 ± 3.20	0.68*
Blood pressure (mmHg)				
Systole	128.27 ± 14.17	126.47 ± 19.02	133.53 ± 13.20	0.22*
Diastole	83.85 ± 5.45	86.47 ± 7.02	82.35 ± 4.37	0.06*

Note. *Independent sample t-test, ** Chi-square goodness of fit test

Pregnant women experience anemia if the iron absorption is obstructed. Gestation at less than 20 or more than 35 years old is more at risk of anemia because they need more iron for themselves and the fetus. Pregnancy under 25 years old is not optimal mentally and biologically, resulting in a lack of attention to iron fulfillment. Meanwhile, gestation over 35 years is more at risk of anemia because of declining reproductive organs (Rejeki & Huda, 2014). The previous study shows that there is a significant relationship ($p < 0.05$) between iron deficiency and pregnancy week, and the

risk of anemia is increasing in the third trimester (Abiselvi *et al.*, 2017; Nasyidah *et al.*, 2013). As the pregnancy age gets higher, the demand for iron supply is also raised. The erythrocytes level in pregnant women reaches its peak at 20 to 25 weeks of gestational age, and most iron deposition in the fetus occurs after 30 weeks of pregnancy. The need for iron during pregnancy and breastfeeding is four times greater (5.6 mg/day) compared to non-pregnancy (1.36 mg/day) (Hallberg & Hulthen, 2000). Many studies have been conducted to determine the relationship between gestational

age and the risk of anemia, and the results were varied (De Camargo *et al.*, 2013; Nair *et al.*, 2016; WHO, 2001).

The Hb level was analyzed before and after 28 days of administration of both

antianemia tablets and in combination with vitamin C. The data can be seen in Table 2.

Table 2. Hb level in antianemia tablet alone group and antianemia-vitamin C group

	Positive control group (n=17)		Percentage (%)	Treatment group (n=17)		Percentage (%)
	Baseline	After 28 days		Baseline	After 28 days	
Hb level	10.89 ± 0.83	10.98 ± 0.79 ^a	0.8	10.64 ± 0.84	11.23 ± 0.76 ^{a, b}	5.5

Note.

Values are presented as mean ± SD

^asignificantly different from baseline ($p < .05$), using paired-sample T-test

^bSignificantly different from the control positive group ($p < .05$), using independent-sample T-test

The Hb levels (gr%) in the positive-control and treatment groups were found significantly increased ($p < 0.05$), which each was raised by 0.8% and 5.5% after four weeks. The analysis of Hb level after vitamin C addition in antianemia supplement revealed a significant increase ($p < 0.05$) value after four weeks. In the initial examination of the Hb level, five pregnant women in the positive-control group and eight in the treatment group experienced mild anemia. A decrease in Hb levels was also seen in six of 14 subjects in the positive control group. Many factors causing this Hb reduction were identified, namely lack of compliance in taking antianemia tablets, lack of antenatal nutritional education, multiple pregnancies, and deficiency of dietary micronutrients intakes (Adam & Ali, 2016; Ayensu *et al.*, 2020), which resulted in disruption of iron absorption (Arisman, 2004).

In general, the Hb levels in Trimester II of pregnancy have decreased due to hemodilution. Blood dilution in pregnancy occurs by 30% to 40% of plasma volume increasing, 18% to 30% of red blood cells escalation, and 19% of Hb decreasing. The physiology of blood dilution is used to ease the burden on the heart. If the average Hb level is around 11 gr%, then the result of hemodilution will be seen in anemia, and the Hb will be 9.5 gr% to 10 gr% (Wiknjastro, 2002). Therefore, increasing Hb levels by consuming iron-rich foods or giving an antianemia supplement is necessary. During pregnancy and breastfeeding, the need for iron is four times greater than usual.

Vitamin C plays a potent enhancer to transform ferric ions into ferrous ions, so it is easily absorbed in higher pH levels in the duodenum and small intestine (Almatsier, 2009).

Iron absorption increases four times with vitamin C combination. In iron metabolism, vitamin C accelerates iron absorption in the intestine and its transfer to the blood. Vitamin C also plays a role in transferring iron from plasma to ferritin. Besides, vitamin C contains a component enriching biological reductants as an essential cofactor of reduction reactions of iron and copper in the function of oxygenation systems, carnitine biosynthesis, and an increase in absorption and metabolism of iron (Jacob & Robert, 2005). Many studies were conducted to evaluate the effect of co-administration of vitamin C in various subjects. Research conducted on children over six years consuming orange juice rich in vitamin C and iron supplements indicates a twofold increase in iron absorption of ferrous fumarate (Balay *et al.*, 2010). In a randomized controlled trial study on iron-deficiency anemia (IDA) patients, oral iron supplements alone are equivalent to oral iron supplements plus vitamin C to improve hemoglobin recovery and iron absorption (Li *et al.*, 2020). A review comparing ascorbic acid (AA) and other organic acids shows that AA increases iron absorption more potently than other organic acids (Teucher *et al.*, 2004). A recent investigation on iron-deficient women indicates that the absorption of oral iron,

particularly when taken with meals, will be higher if given with ascorbic acid, about 6 mg of ascorbic acid for each 1 mg of iron (Stoffel *et al.*, 2020). A combination of antianemia supplements with vitamin C is expected to increase the absorption of iron during pregnancy. The present study indicates that combining antianemia supplements with vitamin C enhances Hb levels compared to antianemia tablets alone in pregnant women's first and second trimesters. However, this study should be confirmed through further investigation due to the small number of patients enrolled. Therefore, prospective clinical research with a more significant number of patients is required to verify these findings.

CONCLUSION

Supplementation of vitamin C in antianemia tablets potentially increases the hemoglobin level compared to antianemia tablets alone in pregnant women's first and second trimester.

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