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# The Role of Addition of Vitamin C in Iron Supplementation on Ferritin Serum Levels in Anemia Adolescent Females

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## ABSTRACT

Prevention of iron deficiency anemia in Indonesia using ferrous sulfas blood-added tablets started since 1997. But iron deficiency anemia is still high, also in young women. Young women are vulnerable because of rapid growth and unhealthy their consumption patterns, which rarely consume fruits and vegetables that are a source of vitamin C. The purpose of this study was to analyze the addition of vitamin C in iron supplementation + folic acid to serum ferritin levels in adolescents females anemia. This research was an experimental quasi research. Design of research was Randomized Pre Post Test Control Group Design, with Double Blind treatment. This study had one treatment group and one control group, each group of 11 samples. The samples were chosen randomly with inclusion and exclusion. The collected data was processed using SPSS program with the analysis of paired t-test and independent t-test to test the difference of serum ferritin level. The results was showed the characteristics of respondents: age ($p = 0.430$), education of mother ($p = 0.942$) and nutritional knowledge of respondents ($p = 0.928$) in the same condition or both groups were had homogeneous characteristics. The pattern and level of food consumption were showed no different between control and traetment groups ($p > 0.05$). Serum ferritin levels showed difference between the two groups after treatment ($p = 0.004$). The study was showed vitamin C had role in increasing iron absorption and fill in storage of ferritin formation.

**Keywords:** Anemia, Vitamin C, Iron supplement, Ferritin

## INTRODUCTION

### Background

Iron deficiency anemia is anemia caused by reduced iron body reserves. This condition can be seen in the presence of decreased transferin saturation, and reduced levels of ferritin or bone marrow hemosiderin[(1)]. The status of iron in the human body depends on the absorption of iron itself. Some of which can increase the absorption of iron is vitamin C contained in fruits, vegetables, such as oranges, guava, kale, spinach, and others. Vitamin C acts as an enhancer because it helps the absorption of iron in the form of non heme, thereby increasing the bioavailability of non heme iron[(2)].

Prevention and control of nutritional anemia in Indonesia starting in 2003 by giving Blood-Added Tablets (BAT). BAT is given to pregnant women and postpartum mother, whereas giving BAT to adolescent females is given after target of pregnant mother and postpartum have been fulfilled. The success of therapy using iron supplements results in rapid reticulocytosis within approximately one week and improvement in hemoglobin levels of approximately 2-4 weeks, which will result in a complete improvement of anemia within 1-3 months[(3)].

The result of Basic Health Research in 2013 was showed that the prevalence of anemia in Indonesia is 21.7% with the proportion of 18.4% male and 23. 9% female. Based on the age group, 5-14 years age group anemia patients was 26.4% and in the 15-24 year age group 18.4%[(4)].

Adolescents are vulnerable because teenagers develop rapid growth (spurt growth), young women experience menstruation every month that causes iron loss. This causes young women vulnerable to iron deficiency anemia because there is an increase in iron demand during growth, blood loss during menstruation. One cause of low levels of hemoglobin is inadequate nutrition intake of body needs. Adolescent nutrition intake

is generally influenced by diet. Eating patterns in young women affected by the attention of young women are high on ideal body shape so that teenage girls often limit their intake of food. Restriction of food intake makes inadequate intake of nutrients including iron.

Inadequate iron intake in adolescents because adolescents have poor eating habits. Teenager eating habits on average no more than three times a day. In addition teens rarely consume fruits. Fitrah (2011) states that as many young women have vitamin C intake less than 89.5%. The Madrasah Aliyah students rarely consume fruits such as oranges, papaya and apples which are a source of vitamin C[(5)]. Most girls experienced protein consumption deficiency (58%), iron (64% and vitamin C (66%)[(6),(7)].

In absorption of iron, vitamin C has its own role in the body. Vitamin C is involved in various phases of iron transport, both cellular and molecular levels (Tarng et al, 2004). Vitamin C functions in Fe metabolism, which is to accelerate the absorption of Fe gut and transfer Fe into the blood, but it also plays a role in transferring iron from plasma transferin to liver ferritin[(8)].

Based on the background and identification of the problem mentioned above, the problem formulation in this study is: "What is the role of addition of vitamin C in iron supplementation + folic acid to serum ferritin and hemoglobin female anemia?" The aimed of this study was to analyze the addition of vitamin C in iron supplementation to serum ferritin levels in adolescent girls anemia.

**Purpose**

The purposed of this study was to analyze the addition of vitamin C in iron supplementation to serum ferritin levels in adolescent girls anemia.

## METHODS

This study was a quantitative study and includes quasi experimental research. The research design was Randomized Pre Post Test Control Group Design, with the treatment of Double Blind. The study was done on two groups, they were one treatment group and one control group as the comparison group. Measurements in this study were conducted before and after treatment for each group.

This research was conducted at SMPN 3 Ngronggot Nganjuk. Initial data collection was conducted in April 2017. Initial data was included data on target characteristics, blood sampling to determine anemia status. Supplementation was done daily for 1.5 months. The treatment group was given supplement Ferous sulfate + folic acid + vitamin C as much as 100 mg and control group was given supplement Ferous sulfate + folic acid. Blood sampling was done twice, before and after supplementation. The Blood sampling was saw serum ferritin and hemoglobin levels. This research was conducted in April-September 2017.

The population of this study was all of students at class 7$^{th}$ and 8$^{th}$ in SMPN 3 Ngronggot Nganjuk District. They were 96 female students. The population was carried out 2-stage screening. The first stage was used the inform consent, which was approval to perform the hemoglobin level test. The second stage was conducted a hemoglobin examination test to obtain anemia sub-populations (Hb <12 g / dl).

The samples were taken from sub-populations by inclusion and exclusion criteria. Inclusion criteria: (1) age of adolescent girls (12-15 years); (2) anemia (Hb 8 - <12 gr / dl); (3) willing to be involved in the study by signed informed consent; (4) respondents were not being menstruated when taking blood samples; and (5) they were not in a state severe pain. Exclusion criteria: (1) Respondents were severely ill; and (2) They would not to continue as research subjects. The sample size was determined by the formula of determined the sample size in accordance with Lemeshow (1997)[(9)]. The sample size was 11 respondents for the treatment group and 11 respondents for the control group. The total number of respondents was 22 students. The samples were toke by technique systemic random sampling, so that young women were represented proportionately with several stages.

Serum ferritin were measured by ECLIA (Electrochemiluminescence immunoassay) method at Prodia Kediri Clinic Laboratory. Hemoglobin concentration was measured by hemocue method. The characteristics of adolescent anemia were included age and nutritional knowledge collected by interview used questionnaires. The pattern of food intake and the dieatary intake of protein, iron and vitamin C were obtained through interviewed by Form Food Frequency and Form Food Recall 2 x 24 hours.

The data was collected and then the processed of checked the truth and completeness. After that done the process of coding and editing. Further data was processed by descriptive and analytic. Data was analysis with SPSS program version 16. The categorical data were presenting in the form of frequency and percentage[(10)], while the numerical data were presenting in the form of mean and standard deviation[(11)].

Statistical analysis to determine the difference of serum ferritin and hemoglobin levels at before and after supplementation in both groups was using paired sample t-test ($\alpha = 0.05$). The difference between supplementation of Fe + folic acid + vitamin C and Fe + folic acid was analyzed using independent t-test ($\alpha = 0.05$). Test of normality of data distribution was done by using Kolmogorov Smirnov normality test.

## RESULTS

Table 1. The characteristic of respondens

| Characteristics | Group | | | | p |
|---|---|---|---|---|---|
| | Treatment | | Control | | |
| | n | % | n | % | |
| Age | | | | | |
| 13 years old | 6 | 54.5 | 3 | 27.3 | 0.430 |
| 14 years old | 4 | 36.4 | 7 | 63.6 | |
| 15 years old | 1 | 9.1 | 1 | 9.1 | |
| Level of Nutrition Knowledge | | | | | |
| High | 2 | 18.2 | 1 | 9.0 | 0.928 |
| Enough | 3 | 27.3 | 5 | 45.5 | |
| Low | 6 | 54.5 | 5 | 45.5 | |

Table 2. Food consumption pattern of respondens

| Food Consumption Pattern | Group | | | | p |
|---|---|---|---|---|---|
| | Treatment | | Control | | |
| | n | % | n | % | |
| Food main + side dish animal + side dish of plant + vegetable + fruit | 0 | 0.0 | 1 | 9.0 | 0.248 |
| Food main + side dish animal + vegetable | 3 | 27.3 | 5 | 45.5 | |
| Food main + side dish of plant + vegetable | 7 | 63.7 | 4 | 36.5 | |
| Food main + vegetable | 1 | 9.0 | 0 | 0.0 | |
| Food main + side dish animal + side dish of plant | 0 | 0.0 | 1 | 9.0 | |
| Frequency of Eat a Day | | | | | |
| 1 time | 1 | 9.0 | 1 | 9.0 | 1.000 |
| 2 times | 5 | 45.5 | 5 | 45.5 | |
| 3 times | 5 | 45.5 | 5 | 45.5 | |
| Consumption of Animal Protein | | | | | |
| Often | 0 | 0.0 | 2 | 18.2 | 0.900 |
| Rarely | 10 | 90.9 | 6 | 54.6 | |
| Very rarely | 1 | 9.1 | 2 | 18.2 | |
| Never | 0 | 0.0 | 1 | 9.0 | |
| Consumption of Plant Protein | | | | | |
| Often | 11 | 100.0 | 11 | 100.0 | 1.000 |
| Rarely | 0 | 0.0 | 0 | 0.0 | |
| Very rarely | 0 | 0.0 | 0 | 0.0 | |
| Never | 0 | 0.0 | 0 | 0.0 | |
| Consumption of Vegetable | | | | | |
| Often | 8 | 72.8 | 4 | 36.4 | 0.778 |
| Rarely | 2 | 18.2 | 7 | 63.6 | |
| Very rarely | 0 | 0.0 | 0 | 0.0 | |
| Never | 1 | 9.0 | 0 | 0.0 | |
| Consumption of Fruit | | | | | |
| Often | 6 | 54.5 | 4 | 36.4 | 0.522 |
| Rarely | 3 | 27.3 | 5 | 45.5 | |
| Very rarely | 0 | 0.0 | 0 | 0.0 | |
| Never | 2 | 18.2 | 2 | 18.2 | |

Table 3. Distribution of respondents based on levels of energy consumption

| Levels of Energy Consumption (% RDA) | Treatment | | Control | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| High | 0 | 0.0 | 0 | 0.0 | 0.371 |
| Enough | 1 | 9.1 | 3 | 27.2 | |
| Low | 0 | 0.0 | 1 | 9.1 | |
| Deficyt | 10 | 90.9 | 7 | 63.7 | |
| Total | 11 | 100.0 | 11 | 100.0 | |

Table 3 was showed that there was no significant different of levels of energy consumption at both groups with probability of 0.371 (p >0.05). The most of levels of energy consumption at both groups were deficyt.

Table 4. Distribution of respondents based on levels of protein consumption

| Levels of Protein Consumption (% RDA) | Treatment | | Control | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| High | 0 | 0.0 | 0 | 0.0 | 0.096 |
| Enough | 1 | 9.1 | 0 | 0.0 | |
| Low | 0 | 0.0 | 2 | 18.2 | |
| Deficyt | 10 | 90.9 | 9 | 81.8 | |
| Total | 11 | 100.0 | 11 | 100.0 | |

Table 4 showed that there was no significant different of levels of protein consumption of both groups with probability of 0.096 (p >0.05). The most of the levels of protein consumption of both groups at the deficit rate.

Table 5. Distribution of respondents based on levels of iron consumption

| Levels of Iron Consumption (% RDA) | Treatment | | Control | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| High | 1 | 9.1 | 0 | 0.0 | 0.948 |
| Enough | 0 | 0.0 | 0 | 0.0 | |
| Low | 0 | 0.0 | 0 | 0.0 | |
| Deficyt | 10 | 90.9 | 11 | 100.0 | |
| Total | 11 | 100.0 | 11 | 100.0 | |

Table 5 showed that there is no significant difference in levels of iron consumption from food of both groups with probability of 0.948 (p >0.05). The most of the levels of iron consumption of both groups at the deficit rate.

Table 6. Distribution of respondents based on levels of vitamin C consumption

| Levels of Vitamin C Consumption (% RDA) | Treatment | | Control | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| High | 2 | 18.2 | 0 | 0.0 | 0.340 |
| Enough | 0 | 0.0 | 0 | 0.0 | |
| Low | 0 | 0.0 | 0 | 0.0 | |
| Deficyt | 9 | 81.8 | 11 | 100.0 | |
| Total | 11 | 100.0 | 11 | 100.0 | |

Table 6 showed that there is no significant difference in levels of vitamin C consumption from food of both groups with probability of 0.340 (p >0.05). The most of the levels of vitamin C consumption from food of both groups at the deficit rate.

Table 7. Average, minimum and maximum value of levels of serum ferritin

| Levels of Serum Ferritin (µg/dl) | Treatment | | Control | | p |
|---|---|---|---|---|---|
| | Before | After | Before | After | |
| Average | 9.54 ± 1.38 | 41.89 ± 13.18 | 9.35 ± 1.39 | 27.58 ± 6.48 | 0.04 |
| Minimum | 7.5 | 23.8 | 7.2 | 17.5 | |
| Maximum | 11.4 | 47.4 | 11.6 | 36.9 | |

Table 7 showed that in the treatment group, there was a significant difference of serum ferritin levels between before and after treatment of Fe + folic acid + vitamin C supplementation with a probability of 0.000 (p <0.05). Likewise in the control group there was a difference in serum ferritin levels between before and after treatment of supplementation of Fe + folic acid with a probability of 0.004 (p <0.05). In this study, independent t-test showed no difference in serum ferritin levels before treatment between treatment group and control group with probability of 0.75 (p >0.05). But there was a significant difference in serum ferritin levels after treatment between treatment group and control group with probabilitis of 0.004 (p <0.05).

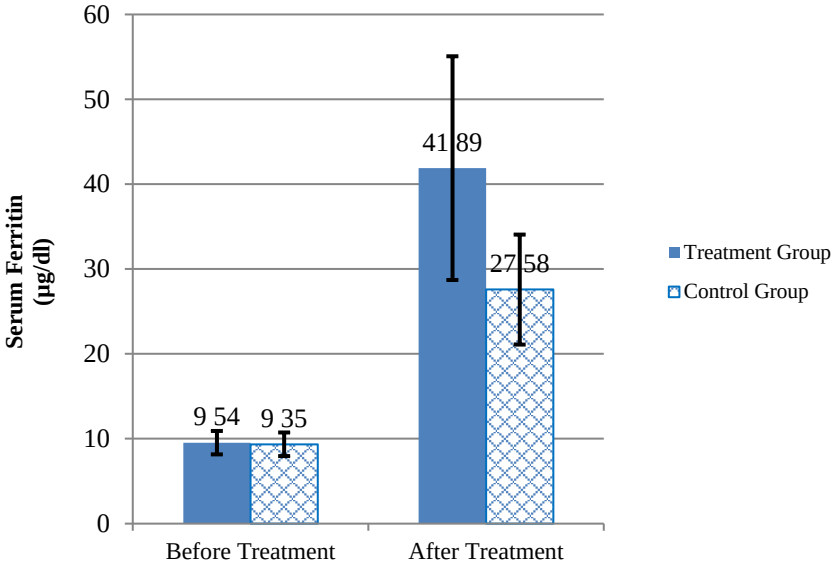


Figure 1. Average of Serum Ferritin in Before and After Treatment

Figure 1 showed different of average of serum ferritin between both groups is as much 14.31 µg/dl more the treatment group. The levels of serum ferritin is matching on both group before treatment.

Table 8. Average. Minimum and Maximum Value of The Levels of Hemoglobin

| The Levels of Hemoglobin (g/dl) | Treatment | | Control | | P |
|---|---|---|---|---|---|
| | Before | After | Before | After | |
| Average | 10.89±0.90 | 13.50±0.99 | 10.89±0.96 | 13.00±0.64 | 0.177 |
| Minimum | 9.2 | 12.5 | 8.9 | 12.1 | |
| Maximum | 11.9 | 15.1 | 11.9 | 14.2 | |

Table 8 showed that in the treatment group and control group, there were a significant difference of hemoglobin levels between before and after treatment of supplement iron with a probability of 0.000 ($p < 0.05$). In this study, independent t-test showed no significant different of hemoglobin levels between treatment group and control group at before and after treatment with each probabilitis of 1.000 and 0.177 ($p > 0.05$).

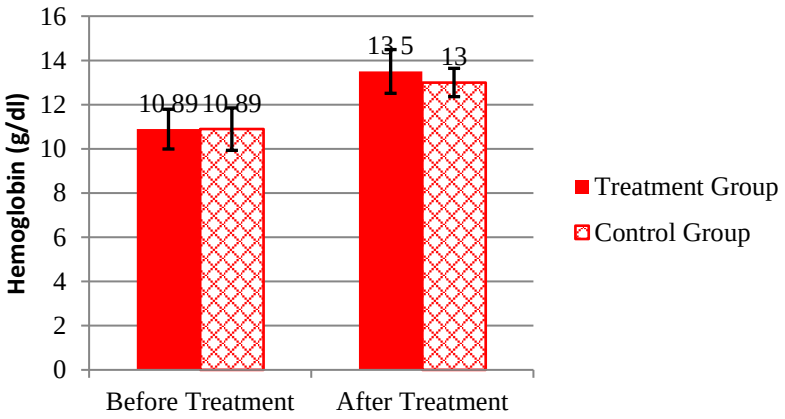


Figure 2. Average of Hemoglobin in Before and After Treatment

Figure 2 was showed average of hemoglobin between both groups was different after treatment. Different of average was 0.5 g/dl more the treatment group than control group. The levels of hemoglobin was as same as on both group before treatment.

## DISCUSSION

Characteristics of respondents include age, mother education and nutritional knowledge of respondents in the same circumstances or in other words both groups in homogeneous characteristics. Age of respondents were ranged from 13-15 years old. Mean age was 13.64 ± 0.81 in the treatment group and 13.82 ± 0.60 in the control group. There was no age difference between treatment group and control group (p = 0.430). The maternal education level and the nutritional knowledge of the respondents were no different from the other groups of treatment and control. Control and treatment group were homogeneous.

According to WHO (2011), adolescents are populations with a age period at 10-19 years old[12]. Kusmiran E. (2011) states that chronologically, adolescents are individuals aged 10-19 years old. In physical terms, the adolescent period is characterized by changes in appearance and physiological features, especially those related to the reproductive organs. While from the psychological side, adolescence is a time when individuals experience changes in the aspects of cognitive, emotional, social, and moral, the transition of childhood to maturity[13].

According to Sukandar (2007), the level of parental education also affects the level of understanding of health care, hygiene and awareness on the health of children and families including in the preparation of food for the family and children.

The nutritional knowledge of the respondents is mostly in the low category. Nutrition knowledge would affect the attitude and behavior of respondents in choosing foodstuffs that it was affected nutritional intake.

The Table of consumption pattern in respondents was showed on the most types of food from both groups was staple food + vegetable side vegetables + vegetables. While the consumption pattern based on eating frequency in both groups is the same. That is between 1-3 times a day meal. The highest frequency of eating a daily from both groups is 2-3 times as much as 90% of respondents. The frequency of good eating is 3 times a day. Frequency 3 times a day to avoid gastric void[14]. The pattern of respondent consumption based on frequency to consume the type of food there was no different in both groups.

Levels of energy consumption, protein, iron and vitamin C respondents from both groups were at the deficit level. The consumption level of both groups before treatment was the same. Ariani (2017) states that protein is necessary in times of growth and for tissue maintenance and also for the manufacture of red blood cells[15]. Protein has a unique function that can not be replaced by other nutrients. Protein in the form of albumin is a protein contained in hemoglobin. Proteins play a role in the formation of hemoglobin because proteins are the main ingredient of hemoglobin formers other than iron[16].

After supplementation there was a marked difference in the number and increase in serum ferritin. This is in accordance with Bakta (2012) which states that if excess iron in meeting the needs of body cells, will be bound by apoferitin into iron storage complex called ferritin[3]. Fe storage for Fe forming red blood cells in the form of ferritin in reticuloendothelial (RE) and hepatocyte cells. Fe that is stored in a small part comes from the result of phagocytosis[17].

Mucosal transferrin transports iron from the gastrointestinal tract into the mucosal cells transferred to receptor transferrin, then transferrin receptors transporting iron (two ferric ions / $Fe3^{+}$) through the blood is brought to the bone marrow (used to make hemoglobin) and all body tissues in need, the excess is stored in the form of ferritin and hemosiderin in the liver (30%), spinal cord (30%) and the remainder in the spleen and muscle[8].

The results was showed the difference in mean serum ferritin levels after treatment between the two groups of 14.31 µg/dl. This study was indicated that the addition of vitamin C increases the absorption of iron in the intestine and can increase the transport of iron thereby increasing the formation of ferritin as iron reserves in the body.

This study were showed consistent with the assertion that vitamins associated with iron deficiency are vitamin C. Vitamin C works in the metabolism of iron, which vitamin C was helped increase and accelerate the absorption of iron in the body or intestines and play a role in the transfer of iron into the blood. The additioned vitamin C was could plays a role in transfer iron from plasma transferin to ferritin of the liver[8],[18].

The results of this study was showed the increased of hemoglobin levels in the both groups. It could been happened because they had the level consume of energy, protein was as same as on deficit level. Therefore to form hemoglobin in erythropoiesis could not optimum. Because in the process need enough of sum protein. However the different of hemoglobin levels between both group was 0.5 g/dl more the treatment group.

## CONCLUSION

The Respondent's characteristic on consumption pattern and level of energy consumption, protein, iron and vitamin C were be same at treatment group and control group before treatment. The addition of vitamin C was increased the absorption of iron in the intestine and transport of iron thereby increasing the formation of ferritin as iron reserves in the body.

To improve iron status in iron deficiency anemia, especially in the increase of iron reserves in the form of ferritin, iron deficiency anemia sufferers are supplemented with iron plus vitamin C. Beside that consumption of energy and protein should be in sufficient quantities and qualities for hemoglobin formation.

For further research can be examined about the appropriate dosage of vitamins C to increase iron status in patients with iron deficiency anemia.

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