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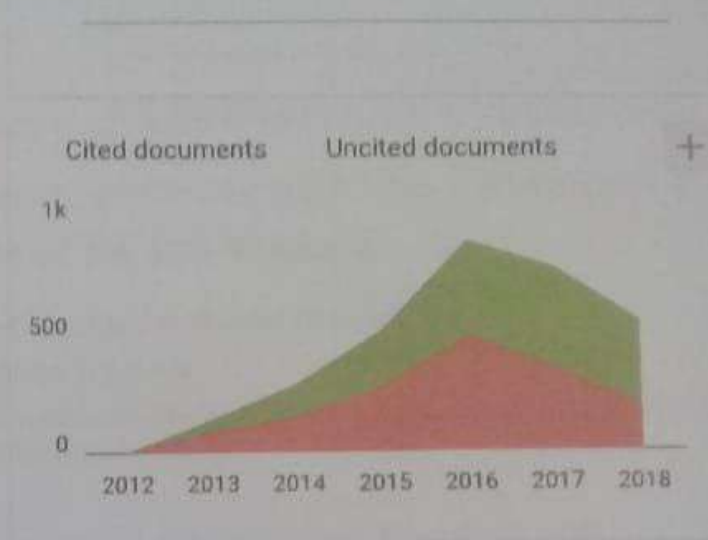
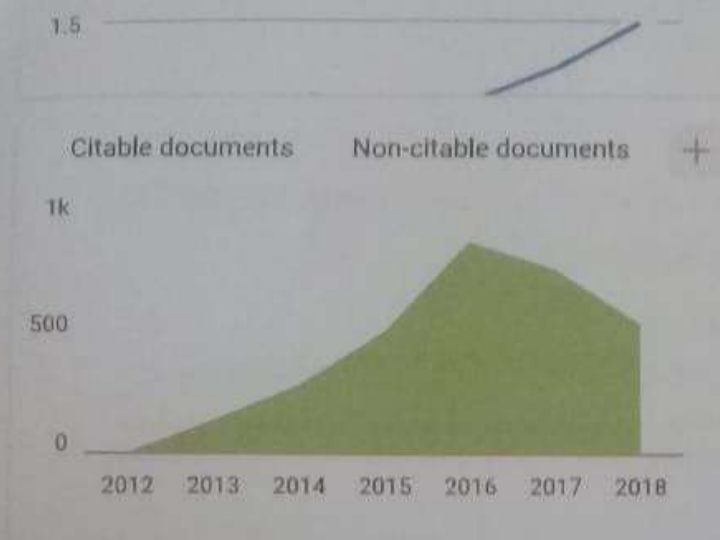
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# Comparison of Hb Levels in Adolescent Girls with The Treatment of The Combination of Fe and Vitamins

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## Abstract

Lower Hb level in anemia is one of the health problems that sometimes occur in adolescent girls compared to males characterized by menstruation cycle and misocundcut of eating behaviors. Reduction of learning concentration and reduction of health and reduction of growth and body weight. The long effect of maternal death, lower body weight of newborn with lower body weight. This present study assesses the effectiveness of supplementation of iron, vitamin C and vitamin A for adolescent girls attending Senior High Schools in Medan, North Sumatera, Indonesia. Sampling technique used stratified sampling method. In Indonesia, the prevalence of anemia is significantly high that achieve 57.1%. This study aims to increase Hb levels of anemic adolescent girls using the treatment of Fe, vitamin C and vitamin A.

The type of the study was experimental using randomized experimental control. The total samples were 140 respondents and samples were divided into three groups. Data were analyzed using t-test and Anova.

Results of the study revealed that the treatment of Fe and vitamin A had higher mean Hb level compared to two other treatment groups.

**Keywords:** anemia, Hb level, Fe, Vitamin A, Vitamin C

## 1. Introduction

Anemia is the condition characterized by inadequate red blood cells and/or hemoglobin in the blood (De, 2001, p.1). No simple classification of anemia can be wholly accurate, but the most useful method is to divide anemia into: (a) microcytic hypochromic or iron deficiency anemia; (b) megaloblastichyperchromic anemia with various forms, such as those due to nutritional deficiencies, but the most important is that known as pernicious anemia; (c) aplastic anemia, a disease in which the red blood corpuscles are very greatly reduced and less than half the cases are due to some toxic substances, such as benzol or certain drugs; (d) haemolytic anemia results from the excessive destruction of the red blood cells and; (e) inherited anemia due to genetic mutation such as sickle-cell anemia and thalassemia (Markovitch, 2005, p. 33).

Symptoms of anemia depend upon whether the anemia is sudden in onset, severe hemorrhage, or gradual. In all cases, however, the striking sign is pallor. The color of the skin may be misleading, except in cases due to severe hemorrhage, as the skin of many Caucasian people is normally pale. The best guide is the color of the internal lining of the eyelid. When the onset of the anemia is sudden, the patient complains of weakness and giddiness, and loses consciousness if he or she tries to stand or sit up. The breathing is rapid and distressed, the pulse is rapid and the blood pressure is low. In chronic cases, the tongue is often sore (glossitis) and the nails of the fingers may be brittle and concave instead of convex (koilonychia). In some cases, particularly in women, the Plummer-Vinson syndrome is present: this consists of difficulty in swallowing and may be accompanied by huskiness; in these cases glossitis is also present. There may be slight enlargement of the spleen, and there is usually some diminution in gastric acidity (Markovitch, 2005, pp. 33-35).

Causes of anemia in developing countries are multi-factorial, which include nutritional (iron, folate, and vitamin B<sub>12</sub>) deficiencies, infections (such as malaria and intestinal parasitic infection [IPI]), and chronic illness (Cheesbrough, 2005). Iron deficiency anemia is the most common nutritional deficiency in the world (the U.S National Academy of Sciences, 2006, p. 336). The world health organizations, declared that, adolescent remain largely neglected, difficult to measure, hard to reach population in which the need of adolescent girls in particular

often ignored (WHO, 2005). The worldwide prevalence of anemia among adolescents is 15% (27% in developing countries and 6% in developed countries) (Balci et al., 2005).

The main important periods in which the need of sufficient and balanced nutrition is the adolescence periods, where growth and development are accelerated (Spear, 2002). The worldwide prevalence of anemia among adolescents is 15% (27% in developing countries and 6% in developed countries) (Balci et al., 2005). The world health organizations, declared that, adolescent remain largely neglected, difficult to measure, hard to reach population in which the need of adolescent girls in particular often ignored (WHO, 2005). The physical and physiological changes that occur in adolescents must be supported by good nutritional needs. Adolescents are at high risk of iron deficiency and anemia. This is due to rapid pubertal growth with sharp increase in body mass, blood volume and red blood cell mass so that they needed iron for myoglobin in muscles and Hb in the blood increases. Iron requirement increases two- to threefolds from a preadolescent level of ~0.7–0.9 mg iron/day to as much as 1.37–1.88 mg iron/day in adolescent boys and 1.40–3.27 mg iron/day in adolescent girls (Usha, 2001; WHO, 2011).

In terms of the specific country level, studies in Indonesia have shown that many adolescents do not come close to meeting intake recommendations for nutrient-rich foods, such as fruit, vegetables, and milk. Therefore, this present study assesses the effectiveness of supplementation of iron, vitamin C and vitamin A for adolescent girls attending Senior High Schools in Medan, North Sumatera, Indonesia. It was implemented by the Medan Health Polytechnic in cooperation with the Regional Health Department of Medan as part of the Nutrition Intervention for Adolescents through Schools.

## 2. Methods of the Study

### 2.1 Material and Method

This research is an experimental research design with quasi experimental design pretest and posttest control group to compare the treatment of Fe, vitamin C and vitamin A at several schools in Medan Derassubdistrict of Batubara regency, North Sumatera Province. Recruitment of the study subjects was conducted by the community outreach method by involving cooperation with staff and undergraduate students of Medan Health Polytechnic as well as enthusiasts at several schools in Medan Deras Subdistrict of Batubara regency and Regional Health Department of North Sumatera Province. Letters that outline the study were mailed to headmasters of schools and staff of Regional Health Department of North Sumatera Province, requesting their cooperation. Invitational flyers were distributed via email to staff, enthusiasts and undergraduate students of Medan Health Polytechnic explaining the research protocol and requesting their assistance in the study.

The locations of the study were conducted at slum areas in Medan Derassubdistrict of the State Senior High School I Medan Deras, the Private Senior High School Citra and the Islamic Senior High School Al-Wasliyah in Medan Deras Subdistrict of Batubara regency.

This study was conducted between February and October 2016 after approval from the Medical Ethical Committee of the Medan Health Polytechnic. This study was descriptive cross-sectional study. Sampling technique used stratified sampling method. The total samples were 215 respondents which classified into three groups of treatment. Hence, each group consisted of 40 samples for each school. However, a 12.5% dropout and hence a total of samples were 105 samples.

### 2.2 Research Procedure

The study used two-group pretest posttest design (before and after intervention) in which each participant is randomly assigned to a group that receives a particular combination of interventions. Group I receives supplementation of 60 mg Fe/day (WHO, 2001) in the form of tablets in simultaneous point in time after meal. Group II receives supplementation of 60 mg Fe/day and vitamin C/day (WHO, 2001) in simultaneous point in time after meal. Each sample in Group III consumed tablets that contain Fe and tablets that contain vitamin A/day in simultaneous point in time. Treatments of all samples were conducted within 4 weeks. Haemoglobin concentrations of the study subject were assessed using the Easy Touch.

Exclusion and inclusion criteria were determined to keep stability of data of the study. (Institute of Medicine of the USA National Academy of Sciences, 2006).

Exclusion criteria:

- a) Individuals with hereditary hemochromatosis, alcoholic cirrhosis (liver disease due to alcohol) and other liver diseases; thalassemias (a group of genetic disorders that involve underproduction of hemoglobin); congenital transferrinemia; and aceruloplasminemia (a genetic disorder that is due to a lack of ceruloplasmin, a protein

that is involved in iron transport) that may have impaired iron absorption.

- b) Individuals with diabetes, heart disease and liver damage.
- c) Individuals with decreased stomach acidity or have pathological conditions (achlorhydria or partial gastrectomy).
- d) Individuals with renal disorders.
- e) Individuals who smoke, abuse drugs or alcohol, or regularly take aspirin.
- f) Individuals under vegetarian diets.

Inclusion criteria:

- a) Aged 17-20 years old.
- b) Hb levels for diagnosis of anaemia for adolescent girls was < 12 gr/dL (WHO, 2005)
- c) A willingness to follow the treatment method in this present study.
- d) Study subjects reside at designated recruitment sites in Medang Deras subdistrict.
- e) Study subject or subject legal representative has signed the informed consent form.

### 2.3 Data Management and Analysis

Analysis of variance (ANOVA) was used to compare the difference between groups in univariate samples and values are expressed as means  $\pm$  s.d. and percentages. In bivariate analysis, Independent samples *t*-test for was used to compare the significance of the difference between two population means before and after treatment of Fe, vitamin C and vitamin A. Hypothesis: If  $t_{\text{calculated}} < t_{\text{table } 0.05 \text{ (critical value)}}$ , then  $H_0$  is accepted and  $H_a$  is rejected, meaning that there is no significant difference between two groups before and after treatment.  $t_{\text{calculated}} > t_{\text{table } 0.05 \text{ (critical value)}}$ ,  $H_0$  is rejected and  $H_a$  is accepted, meaning that there was significant difference between two groups before and after treatment. to assess the effect of of Fe on Hb levels before and after treatment in Group I (48 samples of SMA I), the effect of combination of Fe and Vit C on Hb levels before and after treatment in Group II (48 samples of SMA Citra) and the effect of combination of Fe and Vit A on Hb levels before and after treatment in Group III (44 samples in SMA Alwasliya).

## 3. Results

### 3.1 Univariate Alaysis

Univariate analysis of the study was indicated in the frequency table in the form of descriptive explanation.

Table 1. Frequency Distributions of Hb Levels

Schools	Number of Students before Hb Check	Anemic Students	Percentage
SMAN I	80	48	60%
SMAS Citra	73	48	65%
Aliyah Alwasliyah	62	44	70%
	215	140	

Based on results of the above table, the majority of adolescent girls at senior high schools in Subdistrict Medang Deras had anemia amounted to 140 respondents (65%) had lower Hb levels (<12 gr/dl).

Table 2. Mean Frequency Distribution of Hb Levels at Pretest and Posttest of Total Adolescent Girls

Variable	Mean	SD	Min.-Max.	95% CI
Hb levels (pretest)	10.8	0.57	9.6 – 11.9	10.7-11.0
Hb levels (posttest)	11.5	0.64	10.0-12.9	11.4-11.6

Mean Hb level of respondents at senior high schools in Medang Deras Subdistrict before intervention was 10.8 gr% (95% CI: 10.7-11.0), with standar deviation was 0.57 gr%. The lowest Hb level was 9.6 gr% and the highest

Hb level was 11.9 gr%. From results of estimated data of Hb level, it was concluded that the mean Hb level of respondents in MedangDerassubdsitric was in the interval 9.6 – 11.9 gr% at 95% confidence interval. After the authors of this study conducted intervention, mean value of Hb level of respondents at senior high schools in MedangDerassubdsirict was 11.5 gr% (95% CI: 11.4-11.6) with standard deviation was 0.64 gr%. The lowest level of Hb was 10.0 gr% and the highest Hb level was 12.9 gr%. From results of the estimated interval, it was concluded that mean value of Hb level interval of respondents was 10.0 – 12.9 gr% at 95% confidence interval.

3.2 Bivariate Alaysis

Table 3. Comparison of Mean Value of Hb Levels Before and After Treatment of Fe and Vitamin C.

Variable	N	Mean	PeningkatanHb	SD	SE	p-value
Hb levels (pretest)	37	10.7	0.6	0.55	0.55	0.001
Hb levels (posttest)		11.3		0.37	0.55	

Mean Hb level of respondents before treatment was 10.8 gr% with standard deviation was 0.55 gr%. Mean Hb level of respondents after treatment was 11.3 gr% with standard deviation was 0.37 gr%. The difference value of Hb level before and after treatment was 0.6. Results of statistical test showed that the p-value was 0,001. It was concluded that there was statistically significant difference before and after treatment of Fe and Vitamin C.

Table 4. Comparison of Mean Value of Hb Levels Before and After Treatment of The Combination of Fe and Vitamin A

Variable	N	Mean	PeningkatanHb	SD	SE	p-value
Hb levels (pretest)	35	10.9	0.8	0.70	0.11	0.001
Hb levels (posttest)		11.7		0.57	0.09	

Mean Hb level of respondents before treatment was 10.9 gr% with standard deviation was 0.70 gr%. Mean Hb level of respondents after treatment was 11.7 gr% with standard deviation was 0.57 gr%. The difference value of Hb level before and after treatment was 0.8. Results of statistical test showed that the p-value was 0.001. It was concluded that there was statistically significant difference before and after treatment of Fe and Vitamin A.

Tabel 5. Comparison of The Combination of Fe and Vitamin C and The Combination of Fe and Vitamin A of Adolescent Girls After Treatment

Variable	N	Mean	SD	SE	p-value
Hb levels (pretest)	105	10.8	0.57	0.56	0.001
Hb levels (posttest)		11.5	0.64	0.63	

Results of statistical test showed that comparison of the combination of Fe and Vitamin C and the combination of Fe and Vitamin A of adolescent girls after treatment at senior high schools in MedangDerassubdsirict revealed that p-value was 0.001 with standard deviation after treatment was 0.57 and after treatment was 0.64. It was concluded that there was statistically significant difference before and after treatment of Fe and Vitamin A.

Table 6. Mean Distribution of The Increase of Hb Levels after Treatment of The Combination of Fe and Vitamin C and The Combination of Fe and Vitamin A

Variable	Mean	SD	95% CI	p-value
Fe	11.5	0.64	11.3-11.7	0.02
Fe + Vitamin C	11.3	0.55	11.1-11.5	
Fe + Vitamin A	11.7	0.70	11.5-12.0	



Mean Hb level of respondents after the treatment of Fe was 11.5 gr% with standard deviation was 0.64 gr%. Mean Hb level of respondents after treatment of the combination of Fe and vitamin C was 11.3 gr% with standard deviation was 0.55 gr%. Mean Hb level of respondents after treatment of the combination of Fe and vitamin A was 11.7 gr% with standard deviation was 0.70 gr%. Results of statistical test showed that the p-value was 0.02. Results of the Benferroni analysis showed that there was statistically significant difference before and after treatment of Fe and Vitamin C and Fe and Vitamin A

#### 4. Discussion

Results of the study showed that mean of Hb level of respondents of senior high schools in Medang Deras Subdistrict (SMA Negeri 1, SMA Swasta Citra dan Aliyah Alwasliyah) was significantly still lower ( $\pm 65\%$  of anemic adolescent girls). Many factors cause anemia in developing countries, which include nutritional (iron, folate, and vitamin B<sub>12</sub>) deficiencies and infections (Cheesbrough, 2005). Over nutrition of Fe seldom occurs due to the intake of food, but this is generally caused by Fe supplementation with the symptoms of regurgitation, diarrhea, increased heart pulse, headache, babbling and unconscious mind due to the nutrients in the body utilized by microorganisms (Almatsier, 2004).

Results of interview with respondents revealed that several respondents took lack of iron intake because of an irregular diet and they seldom took breakfast. This is due to their habits to seldom present breakfast. Many respondents drank a glass of tea every day in breakfast and several respondents took lunch according to the lack of menu. Socioeconomic factor was the main reason for the lack of nutrition intake among respondents because the majority of parents of the respondents have occupation as fishermen in Medang Deras Subdistrict.

In addition, the causes of anemia among respondents were caused by the imbalance of Fe metabolism and other micronutrients as well as their interactions in the body through two mechanisms: (i) a specific micronutrient directly absorbs the absorption of other micronutrients, and (ii) deficiency and over intake of a specific micronutrient affects the metabolism of other micronutrients (Ridwan, 2012).

Interactions of micronutrients occur at the phase of absorption of food that contain several micronutrients in the body. Foods contain a mixture of micronutrients that interact each other. At the absorption phase, foods are digested and nutrients are released for the absorption in several tissues and organs. During digestion of foods, a specific micronutrient affects the bioavailability of or absorption of other micronutrients, such as solubility or the regulation of the intestine. Bioavailability of iron increases when this element is consumed together with vitamin C because vitamin C reduces ferri to be ferro that is easy to absorb in the body (Masthalina, 2015).

The side-effects of iron tablets can be reduced if supplements are taken with meals, but absorption is reduced by about 40%. If the supplement is given as a single tablet, it is best ingested at bedtime (Brise, 1962).

Factors that assist the absorption of Fe include amino acids, organic acids (such as vitamin C) and vitamin A. Foods that inhibit the absorption of Fe include legumes that contain phytate, tea and coffee that contain tannins, fruits that contain pectins, spinach that contains oxalate, bayam that reduce the absorption of Fe and therefore this can reduce the absorption of Fe in the body (Almatsier, 2004; Masthalina, 2015). Majority of respondents in the study sometimes take tea that inhibits the absorption of Fe.

Good food sources of heme iron include: beef, pork, chicken, veal, fish such as halibut, haddock, perch, salmon or tuna, shellfish such as clams, oysters and mussels. It is estimated that 85–90% of total iron intake comes from the non-heme form, while 10–15% comes from the heme form (Hurrell & Egli, 2010). In terms of its bioavailability, non-heme iron is absorbed much less efficiently than heme iron. Good sources of non-heme iron include: fortified cereals, rice, wheat and oats, dark green leafy vegetables, dried fruits like raisins and apricots and beans. Heme iron is found in animal foods, while non-heme iron comes from plant sources.

Results of the study revealed that mean Hb level increased after the treatment and showed statistically significant increase at  $p = 0.001$ . Before treatment, mean Hb level of respondents in Medang Deras Subdistrict was 10.7 gram/dL and after treatment was 11.3 gram/dL.

Vitamin C is a water-soluble vitamin that functions as a cofactor for the enzyme required in the hydroxylation of proline and lysine in collagen formation. The highest vitamin C content is found in green and red peppers, broccoli, citrus fruits, strawberries, melons, tomatoes, raw cabbage, potatoes, and leafy greens and mustard greens. Losses of vitamin C occur when foods are cooked in large amounts of water and exposed to extensive heating (The U.S National Academy of Sciences, 1997).

Results of a study showed that Fe increased Hb level after the treatment of 200 mg vitamin C for 60 days for anemic children. Other study revealed also that the treatment of 50 mg vitamin C every day for 8 weeks could

improved that Fe status, however, the treatment of supplementation of 2 g vitamin C for adolescents did not show significant effect of Fe status (Ridwan, 2012). This indicates that treatment of vitamin C will not give a significant effect when individuals lack of food intake as shown also in this present study. This is confirmed for respondents in the present study that many of them did not take regular meals and seldom take breakfast.

A study performed by Fatimah et al., 2011 revealed that anemic pregnant women are associated with the lack of vitamin C intake. This coincides with the study conducted by Tadete A, et al., 2013 that showed the significant relationship between anemic occurrence and the lack of vitamin C intake ( $p=0,042$ ). Results of the present study showed that there was significant increase of Hb level after the treatment of vitamin C, and accordingly, vitamin C has several functions in increasing the absorption of Fe.

Fe is found mainly in animal protein and plant protein. Fe in the form of non-heme is commonly found in plant protein and becomes the source of foods for the developing countries including in Indonesia. Vitamin C increases the absorption of Fe when it is consumed simultaneously with other foods because vitamin C alters Fe to be ferrous. Ferrous is easy to absorb and moreover, vitamin C forms the ferrous-ascorbate group that is soluble in higher pH in the duodenum (Almatsier, 2006)

The above studies proved that the effects of vitamin C increased the status of Fe, not only depend on the status of Fe at the preliminary study, but also it is affected by other nutrients in foods that affects the absorption of Fe. Results of the present study are compatible with the previous studies that the combination of Fe and vitamin C could improve the status of anemia of as proved in their Hb levels after treatment.

Other reasons that the increase of mean Hb level after the treatment of tablets of Fe and vitamin C is the obedience of respondents who took regularly the tablets. In this present study, the increase of Hb level after the treatment of the combination of Fe and vitamin C showed significant increase of Hb level, but could not achieve normal hemoglobin normal ( $<12$  gram/dL). This is due to unregular diet intake of respondents and process of menstruation that resulted in below normal Hb level.

The absorption of Fe are influenced by many factors, Animal protein and vitamin C increase the absorption of Fe. Coffee, tea, calcium salt, magnesium can bind with Fe that reduce the absorption of Fe. Conversely, tablet of Fe consumed with foods can increase the absorption of Fe and therefore the consumption of Fe should not be given in simultaneous time with these diets. On the other hand, the treatment of Fe in the form tablet proved reduced mineral levels such as Zn and serum (Ridwan, 2012).

The Indonesian Government has launched the program of Nutrition for adolescents by giving the tablets of Fe every day for 3 months (Arumsari, 2008). Supplementation of Fe is one of the nutrition strategies to increase Hb level in the blood and the adolescent health when this element is combined with the enhancers of the absorption of Fe. Fe in the form of heme combines with protein and contains in foods particularly in animal protein. More than 35% of the total heme is directly absorbed in the body. Another form of Fe is non-heme as the compound of complex inorganic compound in foods and it is only absorbed 5% in the body. Vitamin C enhances the absorption of Fe in the form of non-heme become fourfold and vitamin C forms ascorbate compound that is soluble when it is absorbed in the body (Yanagishawa et al., 2009). Vitamin C functions in hydroxylation reactions, which affect the function of leukocyte and macrophage function, immune response, wound healing, and allergic reactions (The U.S National Academy of Sciences, 1997)

Vitamin C in food can improve the absorbability of fortification iron that contain inhibitors of iron absorption. The effects of foods containing the same amount of ascorbic acid appear to be the same. Increased absorption of iron can be done by consuming foods that contain about 50–100 mg of ascorbic acid (Hallberg et al., 1987).

Results of the present study showed that for the total samples (44 respondents) had anemia (Hb level below 12mg/dl) although there was statistically significant increase of Hb level. This was proven by independent t-test at  $p\text{-value} = 0,001 (< 0.05)$ . This revealed the significant effect of the treatment of the combination of Fe and vitamin A on the increase of Hb level of anemic respondents. This is in line with the study that the treatment of vitamin A and Fe can interact each other through mobilization of Fe in the liver (Machlindan Langseth, 1998).

Symptoms of anemic persons due to vitamin A deficiency include keratinised skin, dry mucous membranes, xerophthalmia an irreversible drying of the conjunctiva and cornea and keratomalacia (Sommer, 1982; Katz et al., 1995; WHO, 1997).

Vitamin A supplementation improves hemoglobin concentrations (Staab et al., 1998; Suharno et al., 1993). There is clear evidence of an association between plasma serum levels of vitamin A and hemoglobin levels. Mejia (1992) has reviewed the importance of adequate vitamin A status for effective utilization of iron and for maintaining normal haemoglobin. A study in Bangladesh also showed a significant increase in haemoglobin level of

anaemic girls when vitamin A supplements were added to iron and folic acid supplements (Ahmed et al., 2001).

Other studies suggest that vitamin A supplementation improves hemoglobin concentrations (Staab et al., 1998; Suharno et al., 1993). There is clear evidence of an association between plasma serum levels of vitamin A and hemoglobin levels. Mejia (1992) has reviewed the importance of adequate vitamin A status for effective utilization of iron and for maintaining normal haemoglobin. A study in Bangladesh also showed a significant increase in haemoglobin level of anaemic girls when vitamin A supplements were added to iron and folic acid supplements (Ahmed et al., 2001).

Symptoms of vitamin A deficiency include keratinized skin, dry mucous membranes, xerophthalmia (drying of the conjunctiva and cornea) and keratomalacia (softening of the cornea) (Sommer, 1982; Katz et al., 1995; WHO, 1997). Vitamin A has functions to maintain epithelial cells and mucous membranes, constituent of visual purple (for night vision), necessary for normal growth, development and reproduction and maintenance of immune system.

Vitamin A has many roles in the human body including the growth and differentiation of cells of erythrocyte progenitors, increasing the maintenance of the body to prevent infection and mobilization of Fe stores at all tissues. Interactions between vitamin A and Fe occur synergistically as proven by the treatment of the combination of Fe and vitamin A that lowered the prevalence of anemia and restore the utilization of Fe compared to the single supplementation of vitamin A or Fe only. This is confirmed by a study that the lack of vitamin A affects negatively the transportation of Fe from the liver and the combination of Fe and vitamin A will disturb (Semba, 2002)

Results of the reports of several studies summarized by FAO/WHO 2004, revealed that the lack of vitamin A affects negatively the metabolism of Fe. Interaction between vitamin A and Fe as shown in this present study revealed that the supplementation of vitamin A could increase Hb levels of respondents through their Fe level as proven from the decrease of Hb levels of respondents.

This was proven from results of the treatment that Hb level significantly increased in higher level for respondents treated with the combination of Fe and vitamin A compared to those treated by Fe (Parmaesih et al., 2011). Vitamin A helps regulate the release of Fe from the liver. This is in line with the results of the present study that the treatment of the combination of Fe and vitamin A increased the status of Fe in higher level compared to the supplementation of Fe only.

Results of the present study showed that there was increase of Hb level after the treatment of the supplementation of Fe and vitamin A for weeks had anemia (Hb level below 12mg/dl) although there was statistically significant increase of Hb level. This was proven by independent t-test at p-value = 0.00 (< 0.05). This revealed the significant effect of the treatment of the combination of Fe and vitamin A on the increase of Hb.

Mean value of the increase of Hb level of respondents (1) for the treatment of Fe was 11.5 g/dl, the treatment of Fe and vitamin C was 11.3 g/dl and the treatment of Fe and vitamin A was 11.7 g/dl. This is in line with the previous studies that the treatment of the combination of Fe and other micronutrients increased Hb level such as vitamin A and Vitamin C (Asterina, 2009; Ridwan, 2009; Utama et al., 2013).

Results of the present study coincide with the study that the supplementation of Fe will increase oxygenation in cells to be better level, increase metabolism and function of cells optimally metabolism to increase the absorption of foods (Almatsier, 2006). Fe has essential functions as the transporter of oxygen from the lungs to whole tissues in the body ke jaringan tubuh, to transport electrons in the cells and the integrated reaction of enzymes in the tissues.

Fe in the foods was in the form of heme. Heme is absorbed in mucous cells as the complex of porphyrin ring and then it is separated by specific enzymes (hemoxygenase) and Fe is then released. Fe in the form of non-heme across the same pathway and released from mucous cells using the same transportation mechanism. Absorption of heme is not largely affected by composition of food and secretion of digestive channels and the status of Fe of individuals. Heme is the only small part of Fe derived from consumption of food (5 % of the total Fe in the food), particularly in Indonesia, but this element can be absorbed until 25 % and heme is absorbed only 5 % (Almatsier, 2006).

Vitamin A has functions to maintain epithelial cells and mucous membranes, constituent of visual purple (for night vision), necessary for normal growth, development and reproduction and maintenance of immune system. Symptoms of deficiency include keratinised skin, dry mucous membranes, xerophthalmia an irreversible drying of the conjunctiva and cornea and keratomalacia (Sommer, 1982; Katz et al., 1995; WHO, 1997).

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Results of the comparison of Hb level for adolescent girls at senior high schools in MedangDerassubdistrict showed that the combination of Fe and vitamin A had higher Hb level compared to the treatment of two other groups (Fe and the combination of Fe and Vitamin C). This is because the intakes of foods of respondents in MedangDerassubdistrict contain lower level of vitamin A compared to vitamin C. The treatment of vitamin A could prevent the infection disease because this vitamin has function of the progenitor of erythrocyte that increase immunity and facilitates the absorption of Fe (Ridwan, 2012).

Vitamin A supplementation improves hemoglobin concentrations (Staab et al., 1998; Suharno et al., 1993). There is clear evidence of an association between plasma serum levels of vitamin A and hemoglobin levels. Mejia (1992) has reviewed the importance of adequate vitamin A status for effective utilization of iron and for maintaining normal haemoglobin. A study in Bangladesh also showed a significant increase in haemoglobin level of anaemic girls when vitamin A supplements were added to iron and folic acid supplements (Ahmed et al., 2001). The most specific clinical effect of inadequate vitamin A intake and deficiency is nutritional blindness that includes xerophthalmia, an irreversible drying of the conjunctiva and cornea (Katz et al., 1995; WHO, 1997) and keratomalacia (Sommer, 1982).

Symptoms of toxicity (excessive intake) of vitamin A include dry skin, loss of appetite and hair, enlarged spleen and liver, abnormal pigmentation of skin, and fetal malformations. Risk factors of vitamin A deficiency are preterm delivery, heavy menstrual losses, low intakes of vitamin C and allergy to cow's milk.

## 5. Conclusions

1. Mean Hb level for adolescent girls at senior high schools in MedangDerassubdistrict was abnormal or under normal (<12 gr/dl) before and after the treatment although mean value of their level showed statistically increased in term of Hb level.
2. Supplementation of the combination of Fe and vitamin A interact synergistically that increase Hb levels of the respondents.
3. Supplementation of the combination of Fe and vitamin A interact showed significant effect on the absorption of Fe and could increase Hb level.
4. Mean value of the increase of Hb level was higher in the group treated with the combination of Fe and vitamin A.

## 6. Suggestion

1. It was suggested to perform further studies concerning the intake of nutritious foods, Fe, protein, and other micronutrients to investigate more detail the consumption of foods related to anemia.
2. Further actions to conduct health public services by performing health activities related to the routine Hb check, health counseling related to the intake of diets, the health programs of the intervention of Fe and vitamin C and vitamin A to support the efforts of the Government to prevent anemia among adolescents particularly at senior high schools.

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## Competing Interests Statement

The authors declare that there are no competing or potential conflicts of interest.

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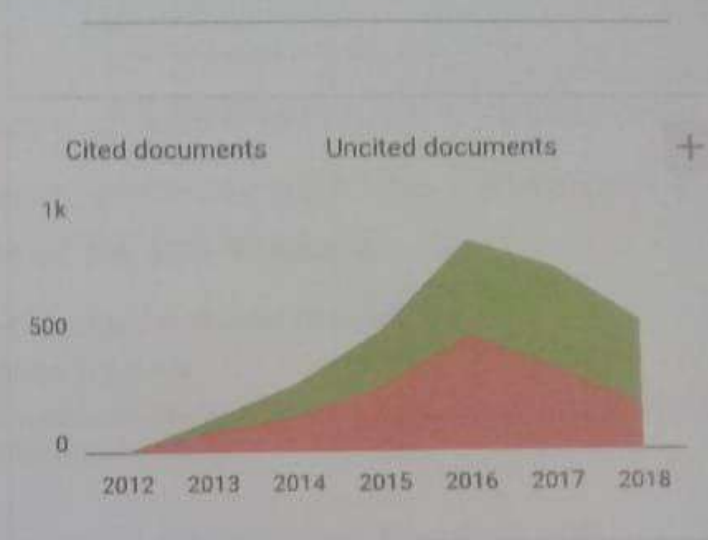
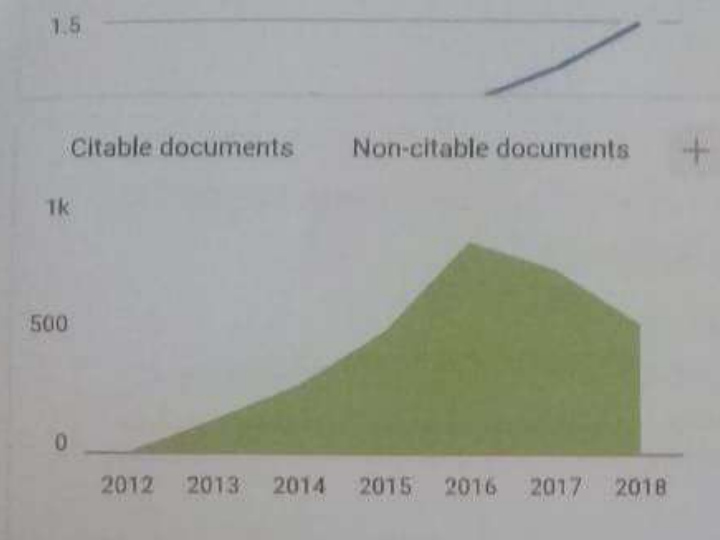
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