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The Efficacy of Multiple Micronutrient Supplementation on Improvement Hemoglobin and Serum Feritin Level in Adolescent Girls with Anemia

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Abstract- The prevalence of anemic adolescent is still public health problem in Indonesia. The objective of this study was to compare the effect of multiple micronutrient and iron folic acid supplementation on hemoglobin and ferritin serum levels in adolescent school girls who suffer from anemia. The study was a randomized controlled trial conducted in five schools in Maros Regency, South Sulawesi Indonesia from January to October 2013. The subjects were 148 adolescent girl with anemia were randomly allocated into two groups. The first group (n=75) received a multiple micronutrient (MMN) and second group (n=73) received an iron folic acid (IFA) supplement. Supplement was consumed twice a week for 26 weeks. The average of hemoglobin levels increased significantly in both treatment groups after supplementation with multiple micronutrient (0.73±1.1 g/dl; p=0.000) and iron-folic acid (0.61±0.98 g/dl; p=0.000), While serum ferritin levels were not significantly increased in both of group, namely multiple micronutrient (2.03±23.2 ng/ml; p=0.420) and iron-folic acid (8.31±31.8 ng/ml; p=0.078). The increased levels of hemoglobin and ferritin serum in two groups did not differ significantly. The prevalence of anemia was significantly reduced in IFA group (25.9%) and MMN group (17.6%). It can be concluded, twice-weekly supplementation with MMNs for 26 wk is not more efficacious than iron and folic acid in improving the hematologic status of anemic adolescent girls. Future studies are needed to increase of the frequency of micronutrient supplementation (3 times a week) with sufficient macro nutrient intake and prevent the incidence of infectious diseases.

Index Terms- multiple micronutrient supplementation, hemoglobin, serum feritin, adolescent girls

I. Introduction

The female adolescent is a crucial period for the woman's life. Health and nutritional status during this phase is important for physical maturity, which in turn will affect the health of the offspring [1]. The nutritional status of female adolescents contributes to the nutritional status of the community[2]. However, the female adolescents are included as one of the vulnerable nutrition groups due to : 1) the more requirement of nutrient because of accelerating the growth and development of the body; 2) changes in lifestyle and eating habits that require adjustment of nutrient intake, 3) pregnancy, active in sports, suffer disease, which increases nutrient requirements[3,4].

Anemia of iron deficiency is the major micronutrient deficiencies that affect the youth particularly in developing countries[5], hence the need of iron to support growth and menstruation often exceeds intake[6]. However, the nutritional anemia is not only caused by iron deficiency, but also by other micronutrients such as vitamin A and folic acid[7,8,9], vitamin B12[10,9], B2[11,12], vitamin C[13,12], selenium[14]. All of which are the precursors to the eritropoiesis in the bone marrow, hemoglobin formation, metabolism, absorption and mobilization of iron in the body[15,16].

The global prevalence of anemia among school-age children was estimated at 25.4%[17]. In Indonesia, based on the data of Riskesdas 2007, there were 19.7% of women (≥15 years old) who suffered from anemia (8% of urban respondents), 59.9% of them suffered from anemia microcytic-hypochromic[18], which was considered as a public health problem based on WHO criteria (20-39.9%)[19].

However, efforts to improve nutrition have been more focused on pregnant women, whereas impact female adolescent are expectant mothers who have to be healthy and give birth the healthy babies. The main barrier in the implementation of prevention and control programs particularly the nutritional problem of anemia in girls, was probably due to lack of knowledge of policy makers on the risks of anemia in girls and low priority in the agenda of nutrition[20]. One way to decide the issue of nutrition and health intergenerational is to improve the nutrition of female adolescent, in other words, female adolescent which is an opportunity to break the intergenerational cycle of malnutrition[21,22]. Therefore, paying attention the nutrition and health of adolescent girls is required.

Micronutrient deficiency problem in female adolescents can be improved through the provision of micronutrient supplements. Based on UNICEF/WHO/UNU recommendation, regarding the use of multiple micronutrient supplement in formula UNIMMAP on females adolescent, it is necessary to investigate multiple micronutrient supplementation in anemic girls to improve hematologic profiles as a regulator eritropoiesis. School-based supplementation programs can be an effective channel of supplementation, when implemented and supervised by the

teacher. The efficacy of multiple micronutrient supplemenetation (UNIMMAP formula) on the female adolescent suffer from anemia nutrient deficiency on the improvement of hematologic profile of female adolescent in Indonesia is still relatively limited, there for this study is urgently required. So, the purpose of this study is to compare the effect of multiple micronutrient (MMN) and iron folic acid (IFA) supplementation on hemoglobin and ferritin serum levels adolescent school girls who suffer from anemia.

II. Material and Method

1.1. Study Participant

The population in this study is all female students who are in class X and XI in 5 Government senior high school in Maros Regency, with the total number of females students are 1158 female students. Government senior high school were selected purposively based on the considering that schools are in a rural area, easy to reach and there is the willingness of the school to participate in the study, evidenced with the acquisition of permission from the school principal, and the school has a relatively large number of females students

Initial screening for anemic (Hemoglobin <12 g/dl) was carried out with a B-Hemoglobin Analyze (HemoCue, Angelholm, Sweden) on a finger-capillary blood sample. Those who were identified as anemic were then invited to provide a venous blood sample, to measured again hemoglobin concentration based on cyanmethemoglobin method as baseline sample. Female adolescent were eligible for the study if they have regular menstruation in every month, non married and pregnant. Subjects were exclude they had severe anemic (Hb <8 g/dl based on hemoCue method), suffering from acute or chronic infections (eg, tuberculosis, typhoid, malaria, dengue fever, etc.), or a metabolic disorder that can affect hemoglobin levels at the time of blood sampling, and had taken the other nutritional supplements (vitamins and minerals). The drop out criteria was the subjects who are resigned from the study or does not participate fully in the research activities in accordance with the research protocol, subjects change schools, lysis of blood taken or damaged so it is not able to be analyzed.

The sample size calculation based on 80% of study power to be able to detect a 5.2 g/dl[23] difference in hemoglobin level between multiple micronutrient and iron folic acid (control)) group, assuming a two tailed test, with α=0.05 and hemoblogin standar deviation (SD) of 10.1 g/dl (based on values observed across all treatment groups)[24]. The number of subjects required in each set was 59 students, and assuming loss to follow up 20% of students, so it required 74 samples per group. The study was conducted from January to October 2013, after obtaining permission from the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar South Sulawesi Indonesia.

1.2. Study design intervention

This study was a randomized, double-blind controlled trial as an experimental trial. The subjects entered into the study were randomly assigned to 1 of 2 supplemented groups: to receive either a double dose of multiple micronutrient (MMN as UNIMMAP formulation containing 15 micronutrient), or iron-folic acid (IFA) as Indonesian Government program for 26. Randomization by using 2 coded groups (A vs B) carried out by an independent research, and female adolescent were allocated to 1 of the 2 codes by computer. All the personnel and investigator were blinded to the 2 groups assignment. Encapsulated of the two supplements is carried out by a pharmacist in the research centre of nutrition and health Hasanuddin University, Makassar South Sulawesi Indonesia. The composition of the two kinds of supplements are presented in the Table 1. Both supplements were identical in appearance and were color coded, were kept by the independent researcher at medicine faculty Hasanuddin University and opened only after data analysis was completed. All participants received the capsule twice weekly for 26 weeks for the benefit of nutritional anemia therapy and re-store the body's iron reserves[25].

Table 1. The composition of the mltiple micronutrients (MMN) and iron-folic acid supplement, nutrient supply from each supplements for female adolescents

Nutrient	Dose	RDA 2013 16-18 years old	Nutrient supply per a day from each supplement
Multiple Micronutrient Supplement (Double doses)			
Vitamin A (Retinol)	800 µg	600 RE (µg)	457.1
Vitamin D (Cholecalciferol)	200 IU (5 µg)	15 µg	2.86 µg
Vitamin E (Tocopherol)	10 mg	15 mg	5.71 mg
Vitamin B-1 (Thiamin HCL)	1.4 mg	1.1 mg	0.8 mg
Vitamin B-2 (Riboflavin)	1.4 mg	1.3 mg	0.8 mg
Niacin (Nicotinamide)	18 mg	12 mg	10.29 mg
Asam Folat	400 µg	400 µg	228.57 µg
Vitamin B-6 (Pyridoxine)	1.9 mg	1.2 mg	1.09 mg
Vitamin B-12 (Cyanocobalamin)	2.6 µg	2.4 µg	1.49 µg
Vitamin C (As. Ascorbat)	70 mg	75 mg	40 mg
Zink (zinc sulphate)	15 mg	14 mg	8.57 mg
Iron (Ferrous fumarate)	30 mg	26 mg	17.14 mg

Copper (Copper sulfate)	2 mg	890 mcg	1.14 mg
Selenium (Sodium selenite)	65 µg	30 µg	37.14 µg
Iodine (potassium iodide)	150 µg	150 µg	85.71 µg
Iron-Folic Acid Supplement			
Iron (ferrous sulfat)	60 mg	26 mg	17.14 mg
Folic acid	250 µg	400 µg	71.42 µg

1.3. Compliance

Supplements were distributed by the teacher to the students, accompanied by field officers, as well as direct observation of the supplements to be swallowed properly by the subject, and then it is recorded by field assistant in the sheet monitoring. If a female adolescent was absent on the day supplement distribution (Wednesday and Saturday), she received supplement on the day she returned to school. Compliance was calculated as the number of capsule eaten divided the number of capsule to receive). For the holidays, the subjects are given supplements based on the numbers of the day that the supplement is consumed, then they are controlled by phone, and at the time the subjects are at school, they are asked about the number of the supplements they have been consumed.

1.4. Data and sample collection

Data of the females student's characteristics: socioeconomic (education, occupation, income) parents, medical history, menstruation which is asked through interviews using questionnaires (questionnaire). Biochemical, medical history and menstruation data were collected twice: before intervention (baseline = t_o) and at the end (t_6) of the intervention in non-fasted female adolescent. For biochemical data, 3 cc of venous mediana cubiti blood of sample using vacutainer anticoagulant with the remainder centrifuged for collection of serum by Prodia officers, and 4 cc venous mediana cubiti blood of using vacutainer without anticoagulant by Prodia officers. All specimen were transported to the laboratory in dry ice and stored below -20^{0}C. The Hb concentration, red blood cell index and white blood cell as a cell immune was analyzed in the Prodia Laboratory Makassar Indonesia as a private laboratory by the immuno-chemiluminescence method,. Serum feritin consentration that measure the number of Fe stores were analyzed by the ELISA method with a commercial kit (Abcam) and the inter-assay is 4.8%.

1.5. Statistical Analysis

All data were analyzed using SPSS for windows. The difference between baseline and endpoint biochemical indicator were tested by Wilcoxon test, whereas the difference biochemical indicator in both treatment groups were analyzed with the U-Mann Whitney. The difference of prevalence anemic and iron depletion was analysed by Mc Nemar Test. ANCOVA test be used to control confounding factor that influenced the group difference. Anemia was defined as Hb level <12.0 g/dl[26] and iron depletion was defined as serum ferritin concentration Nutrient <15 ug/L[27].

III. Results and Discussion

Figure 1 describes the development of the study sample in each school until the study ended. The number of samples at the beginning of the study was 148 students consisting of 73 students from group A and 75 from group B. As the study ended, the number of samples that drop out (DO) was 37 students (25%), which came from the IFA group was 19 students (26%) and 18 students from MMN group (24%). The number of DO subject between the two groups did not differ significantly (p = 0.78). Based on the number of samples until the end of the study (111 students), then the power test of the calculation of the amount of the sample changed from 80% to 77%.

The characteristics of the subjects and their families do not have significant differences between the two treatment groups, so that it can be stated that the study sample was homogenized before being given micronutrient interventions (**Table 2**).

Total of the supplements that should be taken by each subject is 52 capsules. The average number of the supplements taken by the subjects was ±46 capsule with a range between 34-51 capsules. Compliance in the intake of the supplements (> 80%) in the IFA group was lower (85.2%) than the MMN group (87.1%) (chi-square p = 0.811). Adverse events were reported by subjects who acquire IFA supplements namely: head ache, nausea, stomachache, and chest pain.

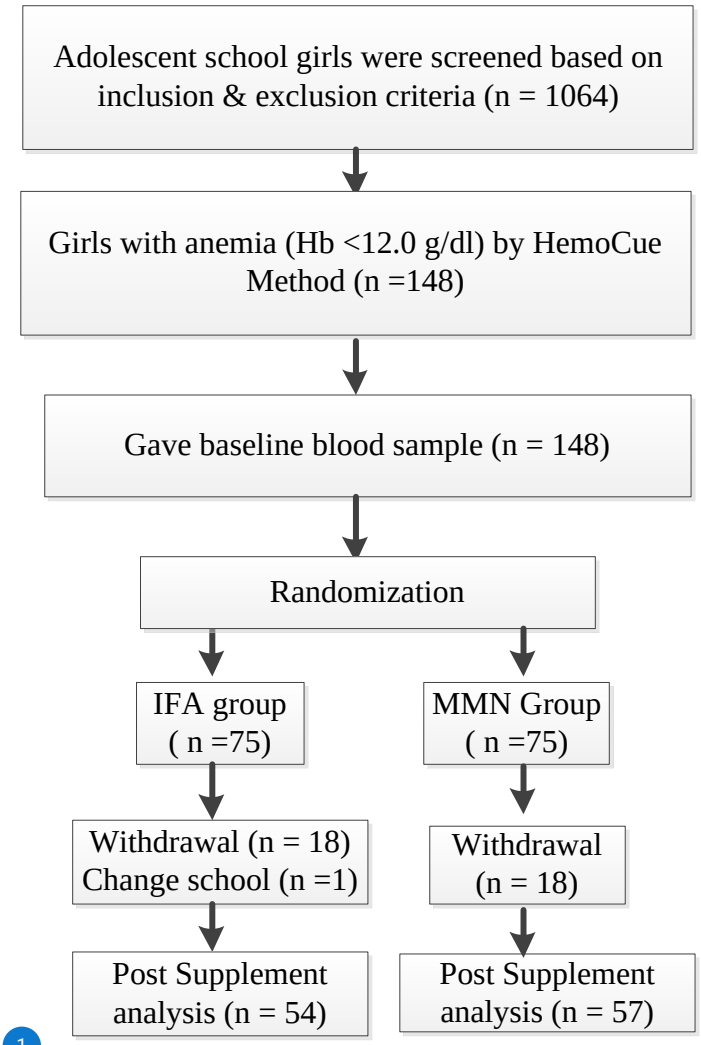

FIGURE 1. Selection process for the study participants and reasons for loss to follow-up in a randomized controlled trial-double blinded with multiple micronutrient (MMN) and iron and folic acid (IFA) supplementation.

Micronutrient intervention gave a significant effect on the changes in hematological parameters. The mean change in Hb values were higher in group MMN subjects compared to group IFA (Table 4). Increased hemoglobin levels were higher in MMN group may be caused by the mean value of serum ferritin and hemoglobin levels before the intervention was lower than IFA group, so that the response rate of MMN group was higher than the IFA group to reach the normal threshold. It is characterized by a rise in the average of hemoglobin level velocity (6.3 g/dl) was higher during the 26-week intervention MMN supplement. Guyton and Hall (2008)[28] states that if there is deficiency of hemoglobin (Hb), then the allotment of more iron for hemoglobin formation, and vice versa when the hemoglobin level is normal, then the allotment of iron components intended for the formation of myoglobin, cytochromes, peroxidase and catalase or stored in the form of ferritin. Supplementation of iron for hemoglobin synthesis is partially absorbed as indicated by elevated levels of hemoglobin, and some go into the tissue to be stored.

Although iron reserves in the body, but without the multiple-micronutrient supplementation, hemoglobin synthesis process is inefficient, and most much iron is absorbed from the intestine to the liver directed. This proves the essential role of multiple micronutrients such as vitamins A, B2, B6, B12, C, Cu, etc., all of which play a role in the synthesis of hemoglobin, either directly or indirectly through a process of absorption and or iron mobilzation. The results of this study demonstrate that functional improvement after supplementation was preferred than sufficient supplies of iron that is characterized by a significant increase in levels of hemoglobin subjects in both treatment groups (p = 0.000).

The increase in mean Hb levels between the two treatment groups were not different significantly (mann whitney p = 0.788) (**Tabel 4**). ANCOVA test results showed that baseline levels of ferritin serum (effect size = 0.176, p = 0.000) was significantly an influential factor to the difference in hemoglobin levels of the subjects (P <0.05), while both types of supplements provide the same effect on the differences in the mean increase in hemoglobin levels (p = 0.820). If iron storage decreased, it's needs 2-3 months to accelarete of absorption to return of normal hemoglobin level[29]. Ferritin serum was a predictor significantly on the hemoglobin concentration in Cambodian children (ß = 2,55; effect size = 0.044; p = 0.011)[30].

If the reduced iron reserves, it takes 2-3 months for accelerating the absorption to restore normal hemoglobin levels (Lynch, 2007)[31]. Serum ferritin is a significant predictor of hemoglobin concentration in children of Cambodia (ß = 2.55 ; effect size = 0.044 , p = 0.01)[32] (Anderson et al., 2008).

These findings confirm the results of previous studies conducted in Bangladesh in girls, namely multiple micronutrient supplementation for 6 months resulted in an increase in hemoglobin concentration as much as 10.5 ± 9.4 g/L (paired T-test p = 0.000) compared to the placebo group increased by only 2.9 ± 6.7 g /L (paired T - test p = 0.000)[33]. Ahmed et.al (2005)[34] reported that the MMN and IFA supplementation 2 times a week for 12 weeks in girls aged 14-18 years found a increase in mean hemoglobin levels for the MMN group (0.63 ± 0:08 g/dl) and the IFA group (0.62 0:08 ± g /dl) were not statistically different (p = 0.91). Hyder study[35] results that provide beverages containing multiple micronutrients on the girls for 12 months giving effect to the increase in Hb 10.8 ± 3.6 g/L was significantly (p = 0.0001) in the first 6 months of intervention. Furthermore, Ahmed et.al (2010)[24] also reported that the IFA and MMN supplementation 2 times a week increase the hemoglobin levels of 8.0 ± 8.2 g /L in the IFA group, and 8.8 ± 9.6 g /L in the MMN group was significantly at week 26 intervention. Study in Bogor found that multiple micronutrient supplementation 3 times a week for 4 months at girls aged 13 -15 years that anemia may increase hemoglobin levels of 15.8 ± 10.7 g/L[23]. In Shaanxi China study that provides multiple micronutrient supplementation (5 mg iron) for 5 months in children aged 10-12 years can increase hemoglobin levels of 3.2 g/L subjects (p = 0.002) and reduced the number of anemia was 12.3 % (p = 0.01)[36]

Table 2. Baseline characteristics subjects and family

Variable	IFA mean±SD	MMN mean±SD	Total mean±SD	P value
Age (years)	16.02±0.8	16.17±0.8	16.09±0.8	0.20[1]
Age of menstruation (years)	12.98±0.9	13.25±0.9	13.12±0.9	0.08[2]
Pocket money (IDR/day)	12648±8077	10710±5654	11653±6975	0.31[2]
Money Buy Snack (IDR/day)	6462±4546	5271±2208	5851±3577	0.58[2]
Family size (person)	5.9±2.0	5.7±2.2	5.8±2.1	0.61[2]
Parent's Income (IDR/mo)	1667124.1±1435854.8	1784210.5±1495429.0	1727249.5±1461785.8	0.62[2]
Education mothers (years)	8.9±4.0	8.3±3.5	8.6±3.8	0.48[2]
Hb (g/dl)	11.72±1.4	11.67±1.3	11.69±1.3	0.92[2]
MCV (fl)	77.92±8.4	78.58±8.2	78.26±8.3	0.68[1]
MCH (pg)	24.59±3.3	25.7±7.2	25.16±5.6	0.46[2]
MCHC (pg)	31.48±1.5	31.51±1.8	31.49±1.6	0.75[2]
Hematocrit	37.2±3.4	36.9±2.6	37.10±3.0	0.51[2]
Serum ferritin (ng/ml)	23.22±23.5	17.27±15.9	20.16±20.1	0.19[2]
Weight (kg)	45.09±7.5	45.02±7.6	45.05±7.5	0.99[2]
Height (cm)	151.31±4.7	151.71±4.9	151.5±4.8	0.67[1]
Height-for-age (z-score)	-1.69±0.7	-1.59±0.7	-1.61±0.7	0.76[1]
BMI-for-age (z-score)	-0.52±0.9	-0.58±0.8	-0.55±0.9	0.77[1]

[1]Uji T Independent [2]Mann-Whitney

On the contrary, based on hemoglobin status, it was still found 28.8% of the subjects suffering from anemia, which was 27.8% in the subjects of IFA group and 29.8% in the subjects of MMN group. It is probably due to several factors other than iron deficiency such as infections that can contribute to blood loss and the loss of nutrients, etc. The results of the study in Bangladesh supporting these findings, is the prevalence of anemia in girls who have obtained multi micronutrient supplementation 2 times/week was found approximately 29.3% and 40.9% in those who acquire iron + folic acid supplements[24]. Besides that, the percentage of subjects who suffer from microcytic-hypochromic anemia decreased significantly to 46.3% (Δ = 25.9%; McNemar test p = 0001) on the subject of the IFA group, whereas the MMN group of subjects was reduced significantly to 36.8% (Δ = 36.9%; McNemar test p = 0.000).

Based on the anemic degree, the percentage of the subjects in IFA group suffering from mild anemia decreased by 22.2% while the subjects in MMN group decreased by 10.5%. Moderate anemia was reduced more in the MMN group subjects (5.3%) compared to the IFA group (1.9%). Severe anemia was not found in the subjects of MMN group while the subjects of IFA group was found to be 1.9% (**Tabel 3**).

Rate of decline in the prevalence of anemia in all subjects after obtaining micronutrient interventions for 26 weeks was 21.7% (McNemar test, p = 0.000). The decrease in the prevalence of anemia in the IFA group was 25.9% (Mc Nemar test, p = 0.003) greater than the MMN group subjects only decreased by 17.6% (McNemar test, p = 0.041) (**Table 3**). The cure rates of anemia differ between subject groups IFA and MMN, which is the cure rate of anemia was higher in the IFA group (48.3%) compared to subjects MMN group (37.0%).

The effectiveness of micronutrient supplementation on anemia status was also seen after the intervention that is the subject of anemia become normal after the intervention in group A (31.5%) and group B (26.5%), while that remained anemic after the intervention in group A (40.7%) and group B (43.9%). The effectiveness of both types of supplements are for improving the status of anemic subjects was statistically significant, namely group A (p = 0.003) and group B (p = 0.041).

Table 3. Iron status before and after supplementation between groups

Indicator	IFA [n, (%)]		MMN [n, (%)]		Total [n, (%)]	
	Before	After	Before	After	Before	After
Hemoglobin Status*	**11.73±1.4**	**12.3±1.4**	**11.67±1.3**	**12.40±1.1**	**11.70±1.4**	**12.37±1.2**
• Normal (≥ 12 g/dl)	25 (46.3)	39 (72.2)	30 (52.6)	40 (70.2)	55 (49.5)	79 (71.2)
• Mild Anemia (11.0 – 11.9 g/dl)	24 (44.4)	12 (22.2)	21 (36.8)	15 (26.3)	45 (40.5)	27 (24.3)
• Moderate Anemia (8.0 – 10.9 g/dl)	3 (5.6)	2 (3.7)	5 (8.8)	2 (3.5)	8 (7.2)	4 (3.6)

• Severe Anemia (< 8 g/dl)	2 (3.7)	1 (1.9)	1 (1.8)	0 (0.0)	3 (2.7)	1 (0.9)
Serum Feritin Status **	**23.2±23.5**	**31.53±28.9**	**17.26±15.9**	**19.29±17.5**	**20.16±20.1**	**25.24±24.4**
• Normal (15 – 150 µg/)	29 (53.7)	35 (64.8)	21 (36.8)	25 (43.9)	50 (45.5)	60 (54.1)
• *Iron depletion* (<15 µg/)	25 (46.3)	19 (35.2)	36 (63.2)	32 (56.1)	61 (55.0)	51 (45.9)

***WHO, 2011a; ** WHO, 2011b**

Besides the impact of micronutrient interventions on the increase of Hb level of the subjects, it is also seen in the increase in the level of ferritin serum of the subjects who indicated the increase in nutrient reserve after receiving micronutrient supplementation. The level of ferritin serum after micronutrient interventions appears higher in the subject of IFA group than MMN group which is significantly different (p<0.05) (**Tabel 4**). Correlation test results showed that the level of ferritin serum endline of the subjects in group B is significantly correlated with baseline levels of eosinophils (r= -0264, p=0.047), baseline levels of basophils (r = -0316, p=0.017). Ferritin serum increased by the possibility of the presence of inflammation and infection, so the correlation of ferritin serum with total body iron becomes less reliable[37]. When the presence of significant inflammation, the levels of ferritin serum do not accurately reflect iron reserves [38]. Ferritin serum in addition to biomarkers Fe reserves in body also serves as a marker of inflammation (acute phase protein) that increased during the acute phase response due to infection[39]. Several types of white blood cells measured in this study, can be used as a marker of inflammation, which is also recommended for the assessment of immune function in public health intervention trial[40]. Although inflammatory markers is significantly correlated to the levels of feritin serum of the endline of group B subjects , but ferritin serum is still subject to normal values within the limits set by the WHO (2011b)[27] is ≤ 150 mg/L. In addition, an increased number of white blood cells as the immune response is only around 1.7 % - 11.1 % (data not shown), which suggests that the prevalence of infection is low[41]. Another explanation is the inflammatory and immune response is most pronounced in body tissues, not in blood, and blood and tissue production may not be relevantly correlated[42]. Therefore, the results of this study indicates that the increase in serum ferritin is an indication of Fe reserves in body.

The difference in mean of serum ferritin levels in the IFA group was 4 times higher than the MMN group, however, statistically it is not significantly different (p> 0.05). Similarly, the increase in serum ferritin levels are also higher in IFA group (1.96 ± 5.1 g/dl) compared to MMN group (0.94 ± 2.4 g/dl), and statistically (Mann Whitney test) the difference between those two groups is not significant (p = 0.46). Higher increase on iron reserves in the IFA group subjects occurred because the mean of hemoglobin level of the subjects after intervention was sufficient to meet the functional needs of the subject's body, so that the rest can be saved as a backup, while on the subject of MMN group, effectiveness of micronutrient supplementation for 26 weeks is only able to improve the profile hemoglobin and red blood cell indices to meet the functional needs of hemoglobin and red blood cell indices, whereas the mean levels of iron is slightly stored. However, the differences in mean level of ferritin serum between the two treatments are not statistically different. The resut of ANCOVA test indicate that the increased levels of ferritin serum of the subjects is simultaneously influenced by hemoglobin levels (p = 0.000) and HAZ-score baseline (p = 0.016) of the subjects before the intervention was significant, while both types of supplements provide the same effect on the difference increase in mean of ferritin serum levels (p = 0411). HAZ-score value was higher in group B subjects (-1.59±0.7) than in group A (-1.63±0.7) which gives an indication that there was a tendency of the nutritional status of the subjects in group B is better than in group A.

The impact of the intervention on the ferritin status of the subjects is shown in **Table 3** in which the percentage of subjects that had a decline in the deficit of iron stores (iron depletion) was 11.1% in group A but not significant according to the McNemar test (p = 0.263), whereas in group B it was decreased by 7.1 % much lower than the IFA group subjects, and a non-significant decline as well based on the results of the McNemar test (p = 0.523).

The result of this study is supported by the results of the study of Ahmed et.al (2005)[34] who reported that multi-micronutrient (MMN) supplementation and iron - folic acid (IFA) 2 times a week for 12 weeks in girls aged 14-18 years found increased levels of serum ferritin were not statistically different (p = 0.89) in both treatment groups, namely the IFA group increased serum ferritin of 5.2 ± 1.3 ng / ml and 5.4 ± 1.3 MMN group ng /ml. The study result of Hyder *et.al* (2007)[35] that gives a drink containing multiple micronutrients to girls who had consumed it for 6 days / week for 12 months showed a significant impact on the increase in serum ferritin levels of 12.5 mg/L (p = 0.001) at the first 6 months of the intervention . The study of Ahmed et. al. (2010)[24] also showed that serum ferritin concentrations in the MMN group and IFA 2 times a week significantly increased at week 26 of intervention, but the increased levels of serum ferritin subject MMN group (19.7 ± 14.7 mg/L) is significantly (p = 0.045) smaller than the acid + iron group folate (24.2 ± 18.8 mg /L) was significantly.

Overall, the result of this study indicates that after consuming micronutrient supplementation in both treatment groups, some iron is used for the synthesis of hemoglobin, which is indicated by increased hemoglobin levels, and partly is stored as reserves indicated by elevated levels of serum ferritin. Increased levels of hemoglobin-related subjects with serum ferritin concentrations before intervention, and vice versa elevated levels of serum ferritin subjects related to serum ferritin levels before intervention.

Table 4. Hemoglobin and serum ferritin concentration before and the end supplementation

Variable	IFA group (n = 54)	MMN Group (n =57)	P Value
Hemoglobin (g/dl)			
baseline	11.73±1.4	11.67±1.3	0.920[2]
endline	12.34±1.4	12.40±1.1	0.930[2]
mean difference	0.61±0.98	0.73±1.1	0.788[2]
P value	**0.000**[1]	**0.000**[1]	
Serum Ferritin (ng/ml)			
baseline	23.2±23.5	17.3±15.9	0.198[2]
endline	31.5±28.9	19.3±17.5	**0.024**[2]
mean difference	8.31±31.8	2.03±23.2	0.344[2]
P value	0.078[1]	0.420[1]	

In conclusion, twice-weekly supplementation with MMNs for 26 wk is not more efficacious than is supplementation with iron and folic acid alone in improving the hematologic status of anemic adolescent girls. So, the findings of the present study have considerable implications for reducing anemia among anemic adolescent girls and support to choose one of them supplement giving twice weekly containing 60 mg iron/dose. Future studies are needed to increase of the frequency of micronutrient supplementation (3 times a week) with sufficient macro nutrient intake and prevent the incidence of infectious diseases.

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