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EFFECT OF PRECONCEPTIONAL SUPLLEMENTATION OF MULTI-MICRONUTRIENTS ON HEMOGLOBIN LEVEL DURING PREGNANCY

***Sri Sumarmi*[1], Soenar Natalina Melaniani[2], Bambang Wirjatmadi[1]**

[1] Department of Nutrion, Faculty of Public Health, Airlangga University
[2] Department of Biostatic and Population Study, Faculty of Paublic Health, Airlangga University
Email : msrisumarmi@gmail.com

ABSTRACT

Introduction: *Anemia during preconceptional period was relatively neglected issues. The other issue is the efficacy of single micronutrient versus multi-micronutrients supplementation.*
Objective: *This study aims to evaluate the effect of multi micronutrient (MMN) supplementation during preconceptional period among the newly married women on hemoglobin level during pregnancy.*
Methods: *A randomized double blind community-based trial had been conducted at Probolinggo District, East Java. Newly married women were recruited as stydy subjects, and randomly assigned into two group: Group I (MMN group) received mutlti micronutrient containing 14 micronutrients 2 other days during preconception, continue with daily dose during pregnancy. Group II (IFA group) received placebo during preconception period and continue with daily iron & folat during pregnancy. Total of 420 eliglible subjects were enrolled to meet approximately 120 pregnancy. The outcome variables is level in week 12, week 28 and week 35 of pregnancy. Social economic background, body mass index, and blood pressure also being assessed as base line data. Nutrients intake also been assesed in each trimester. Base line data was analyzed by using independent-t test and multivariate analysis by general linear model to compare mean differentiation between MMN group and IFA group. Statistical analysis to hypothetical testing has been done to examine effect of treatment on hemoglobin level of MMN group and IFA group.*
Result: *The baseline characteristic between two groups were not different, indicating that two groups are comparable (Box's M value = 44.95 and p = 0.65; Hotelling's Trace T^2 value = 0.094, F = 1.098 and p = 0.371). There were no significant different hemoglobin level in week 12 (p=0.191), week 28 (p=0,655), and week 35 (p=0,865) between MMN group and IFA group. The average of hemoglobin level at last pregnancy (week 35) more than 11 g/dL in both groups.*
Conclusion: *Prolonging supplementation of MMN during preconception will protect anemia or decreasing hemoglobin level less than 11 g/dL as affective as IFA supplementation at last pregnancy, although it still can not increase the hemoglobin level for non anemic women before pregnancy.*

Keywords: *anemia, hemoglobin, preconception nutrition, multi-micronutrients, pregnancy*

INTRODUCTION

Anemia is the one of common nutritional problems among pregnant women and women in reproductive age in developing countries, due to low micronutrient status. Most of anemia

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cases in developing countries, including in Indonesia, were suggested due to low intake of iron from food, therefore the rational intervention to combat anemia was by iron supplementation. In fact, as far as the universal distribution of iron pills program have been implemented to reduce the prevalence of anemia in Indonesia, until now the reduce rate of the prevalence was not as effective as the program planed. Even, this program have been supporting by other programs such as diet modification, iron fortification, and parasite control (Gillspie *et al*, 1991).

Although there was a decrease prevalence of anemia in Indonesia, but the prevalence in all age groups was still more than the trigger level of public health problem. There is an increase trend of prevalence of anemia in Indonesia throughout along life cycle. The prevalence of anemia among unmarried women in reproductive age was about 24,5%, and increase become 26,9% among married women, then increase twice become 50% when the women entering pregnancy period, and the highest prevalence of anemia was found among children under five years. Of children under five years, there was the evidence that the younger children were found the higher prevalence of anemia. The anemia prevalence among babies less then 6 months was 61,3%, prevalence among babies 6-11 months was 64,8%, and prevalence begin to decline among children more then 12 months (Atmarita, 2005; Murniningtyas and Atmawikarta, 2006).

The data trend of anemia seems to be viewed as a continuum of maternal micronutrient status throughout life cycle, especially from the preconceptional period through lactation, and of fetal and infant dependency on adequate maternal status through this time. It's also reflects the intergenerational cycle of anemia. The high anemia prevalence among pregnant women may be caused by they were anemic before pregnant, and the high prevalence of anemia among less then 12 months babies may due to low maternal iron status. It was suggested by De Pee *et al* (2002), that highest prevalence of anemia was found among babies 3-5 months who delivered by the anemic mother. Based on these data and according to Allen (2005), anemia in Indonesia actually should be viewed as a continuum of 3 stages in life cycle: preconceptional, pregnancy and postpartum periods. This data also emphasize the importance of iron status before pregnancy, not just during pregnancy.

Beside of intergenerational cycle problems, it may indicate that anemia in Indonesia does not caused by single nutrient deficiency. It is a rationale reason, because biosynthesis of hemoglobin in the human body is not only need iron alone, but also need the other micronutrients that involve in iron metabolism, such as vitamin A, zinc, vitamin B, vitamin C and cuprum (Devlin, 2001). Vitamin A was needed to produce transferrin, an iron-binding protein that transport iron into the body cells (Semba and Bloem, 2002). Zinc is an important

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compound of *δ-aminolevulinic acid* (*ALA) synthase*, an enzyme that involve in the first step of sequence reaction of heme biosynthesis from glycine and succinyl-CoA which produced by three carboxylic acid (TCA) cycle (Wolfson, 1978). Deficiencies of thus micronutrients can also cause anemia, and iron supplementation alone does not effective to reduce the prevalence of anemia if the other micronutrients deficiencies were not considered.

Based on thus issues, this research focus to evaluate the effect of multi micronutrient (MMN) supplementation during preconceptional period on improving maternal iron status during pregnancy.

METHODS

This is a randomized double blind community-based trial (Altman *et al.,* 2001; Moher *et al.*, 2010). The study setting at District of Probolinggo East Java, was chosen because prevalence of anemia and adverse pregnancy outcome was high in this area (Sri Sumarmi et al., 2008a; Sri Sumarmi *et al*, 2008b). Study subject were newly married women from selected 9 sub districts and randomly assigned into two group: Group I received mutlti micronutrient MMN UNIMMAP formulation (Shankar *et al.,* 2008), it's containing 14 micronutrients 2 other days during preconception, and continue with daily dose during pregnancy. Group II received placebo during preconception period and continue with daily iron and folat (IFA), containing 60 mg iron and 250 μg folic acid only during pregnancy.

Sample size was calculated base on a sample fomula (Pocock, 1983). A sample size of 46 was required for each group on the basis of mean size effect 3.7 g/L and SD 1.7 of hemoglobin level (Muslimatun *et al.,* 2001). By considering the proportion of newly married women with time to pregnancy less than 7 months was 45% (Nurdiati, 2001), we need to enroll 102 subjects each group and when we allow 20% of loss of follow, then need 128 newly married women are required in each group. Total eliglible subjects enrolled in this study were 256. Randomization has been carried out by using a random permuted blokcs method with block size of 10, and allocation ratio 1:1, then we have equal subjects of 128 each group (Meinert, 1986).

The primary outcome variable is maternal hemoglobin level, at week-12, week-28 and week 36. Hemoglobin was determined from sample of approximately 10 μL capilary whole blood that drawn with HemoCue Hb 201 microcuvettes, analyzed using a protable hemoglobin meter kid HemoCue® AB, Anglehome, Sweden). Hemoglobin concentration being colected fourtimes, at enrollement of participants as baseline data, early pregnancy (week 12th) and week 28th of pregnancy as midline data and week 36th of pregnancy as endline data. Variable of Hb

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then categorized as dichotomous data using single cut off point 12 g/dL (before pregnancy) and 11 g/dL (during pregnancy). Social economic background, nutritional status including body mass index and mid uper arms circumferences (MUAC), dietary intake, and blood pressure also being assessed as base line data. BMI was expressed as weight (kg) divided by the square of height (m). Body weight is measured by using digital body scale Seca® type 803, with increment of 100 g. Height or stature is measured by using microtoise with 0.1 cm increment. MUAC was measured by using fiber tape (Unicef), with increment of 0.1 cm. Dietary intake was determined using 24 hours dietetic recall to calculate the daily intake of energy, protein, some vitamin and minerals. Interview using questionares and supporting tools such as food model and house hold utensil will be carried out four times at enrollement of participants as baseline data, early pregnancy (week 12^{th}), week 28^{th} of pregnancy and week 36^{th} of pregnancy. Data of nutrient intake was analyzed using sofware Nutrisurvey, and the intake value then being compared to the Indonesian recommended dietary allowance.

Base line data was analyzed by using independent-t test to compare mean differentiation between treatment group and control group. Statistical analysis using t test to hypothetical testing has also been done to examine effect of treatment on hemoglobin level at week-12, week-28 and week-36 using.

RESULTS

Subjects Enrollment

To cover 256 eligible subjects, we screened out of 978 newly married women. Of the entire subjects who screened, there were 519 newly married women who did not desire to have a child in the first year and use contraception were excluded. Then 459 newly married women who want to have a child in the first year of marriage were continue to the next phase. In the early selection by midwives, 123 married women were not eligible for next phase because they getting pregnant base on rapid test for human chorionic gonadotropin in urine for detecting pregnancy (77) or did not stay at study areas (46). The next step then 336 married women had physical examination and pregnancy test. Of subjects who continued to physical examination, there were 80 women were not eligible since 3 women had tuberculosis infection, 2 women suspect of diabetes mellitus and 75 women were already pregnant. Therefore 258 women were eligible to enroll the study (Figur 1).

All the eligible subjects then randomly assigned into two groups (MMN group and IFA group). In MMN group there were total of 95 pregnant subjects, which are 38 subjects getting

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pregnant before 2 months of intervention, and 57 subjects were pregnant after received 2 months of intervention. Total pregnant subjects in control group were 106, which are 48 subjects getting pregnant before 2 months of intervention, and 58 subjects were pregnant after received 2 months of intervention. Subjects who pregnant before 2 months of intervention were not intensively follow up, and subjects who pregnant after received 2 months of intervention were intensively follow up. Figure 1 performs the flow chart of entire eligible subjects who enrolled and randomized into two groups.

Characteristics of Treatment (MMN) Group and Control (IFA) Group

The social economic characteristic of the subjects were social economic status, marriage status, such as age, education level, occupation and income. Data will be discussed descriptively and compared between MMN group and IFA group.

Tabel 1. Social economic characteristic of MMN group and IFA group

Characteristics	MMN Group (n = 57)	IFAGroup (n = 58)
Age		
< 20 years	25 (43.9%)	15 (25.9%)
20 – 25 years	25 (43.9%)	30 (51.7%)
> 25 years	7 (12.3%)	13 (22.4%)
Education level		
No schooling at all	1 (1.8%)	0 (0%)
Elementry school	8 (14.0%)	9 (15.5%)
Junior High school	13 (22.8%)	16 (27.6%)
Senior High school	29 (50.9%)	19 (32.8%)
Graduate of university/collage	6 (10.5%)	14 (24.1%)
Occupation		
No occupation	39 (68.4%)	37 (63.8%)
Teacher	4 (7.0%)	12 (20.7%)
Health practices	2 (3.5%)	1 (1.7%)
Civil servant	1 (1.8%)	0 (0.0%)
Industrial worker	8(14.0%)	5 (8.6%)
Others	3 (5.4%)	3 (5.1%)
Income		
< 500,000	9 (50.0%)	13 (61.9%)
500,000 – 1,000,000	5 (27.8%)	8 (38.1%)
> 1,000,000 - 2,000,000	3 (16.7%)	0 (0.0%)
> 2,000,000	1 (5.6%)	0 (0.0%)

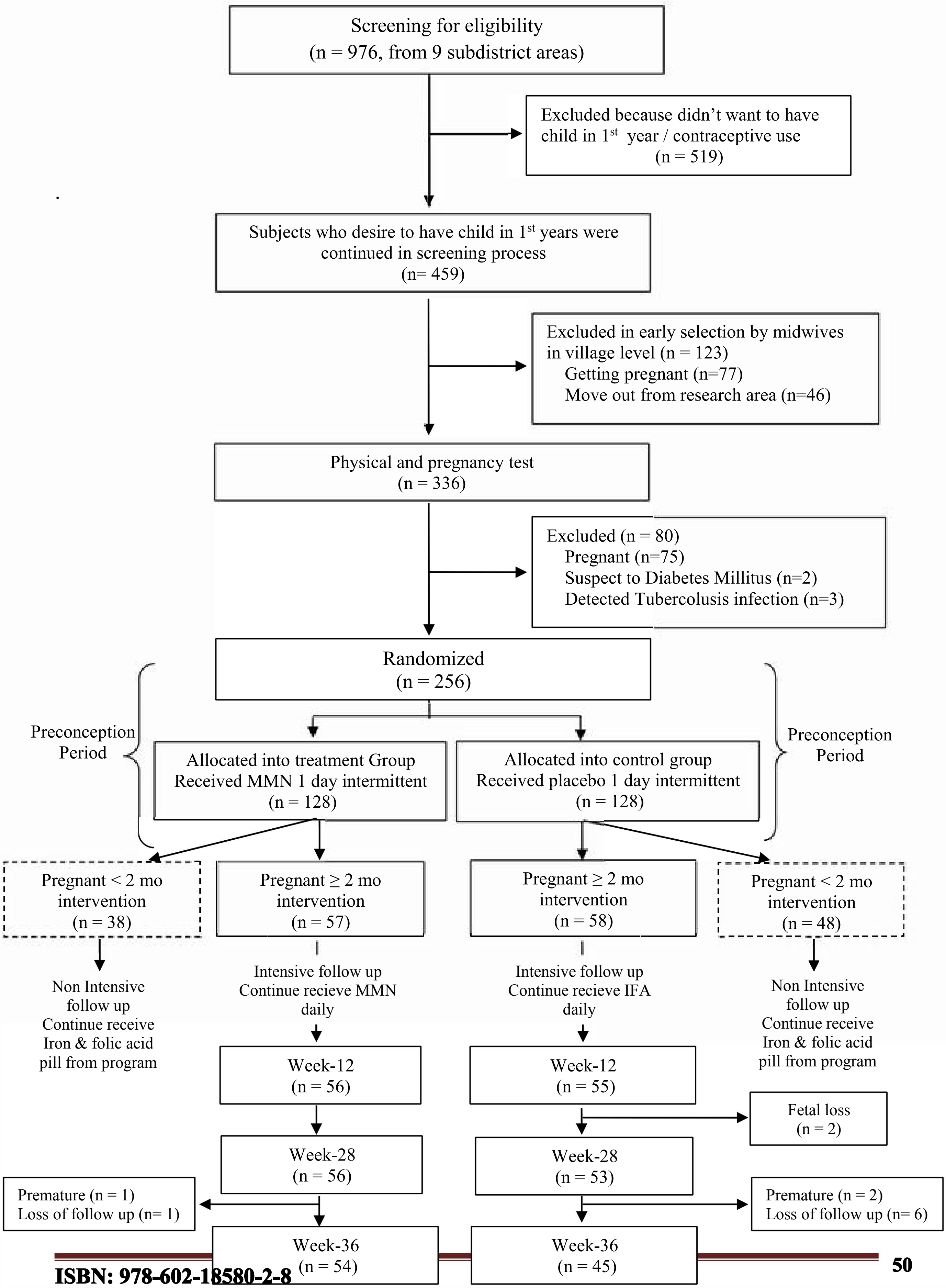

Figure 1. Flow chart of subjects

Baseline Data of MMN Group and IFA Group

The baseline data of primary variable between MMN group and IFA group are compared descriptively. The primary variables discussed are nutritional status including body size such as body weight, height, MUAC, as well as blood pressure and blood glucose and hemoglobin level. Those data represent preconceptional condition among MMN group and IFA group. More detailed data are performed in table 2.

Table 2. Baseline data of MMN group and IFA group

Nutritional Status	MMN Group n=57	IFA Group n=58
BMI		
Under weight	13 (22.8%)	14 (24.1%)
Normal weight	39 (68.4%)	35 (60.3%)
Over weight	4 (7.0%)	7 (12.1%)
Obese	1 (1.8%)	2 (3.4%)
Body Weight		
< 40 kg	11 (19.3%)	10 (17.2%)
≥ 40 kg	46 (80.7%)	48 (82.8%)
Height		
< 145 cm	8 (14.0%)	10 (17,2%)
≥ 145 cm	49 (86.0%)	48 (82.8%)
MUAC		
< 23.5 cm	18 (31,6%)	19 (32.8%)
≥ 23.5 cm	39 (68.4%)	39 (67.2%)
Hemoglobin		
< 12 g/dL	21 (36.8%)	17 (29.3%)
≥ 12 g/dL	36 (63.2%)	41 (70.7%)

Statistical test to compare the homogenity of preconception data between MMN group and IFA group

Univariate analysis for mean comparation between treatment group and control was done using independent sample t test. All preconception data which reflect the initial condition between two groups were tested. Result showed in table 3.

Table 3. Univariate analysis to compare the mean value of several preconception data between MMN group and IFA group using independent t test.

Variables	MMN Group n=57	IFA Group n=58	p value
Age	20.9 ± 3.3	22.4 ± 3.9	0.026
Body Weight	47.4 ± 8.1	47.3 ± 9.2	0.920
Stature	150.9 ± 5.6	149.9 ± 5.7	0.308
MUAC	25.2 ± 3.3	25.3 ± 3.8	0.833
BMI	20.8 ± 3.5	21.0 ± 3.8	0.805
Systole	106.2 ± 8.9	106.2 ± 9.9	0.978
Diastole	70.7 ± 8.7	70.1 ± 8.4	0.727
Hb	12.2 ± 1.1	12.6 ± 1.2	0.112

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Nutrient intake was analyzed separately. Comparation of nutrient intake between MMN group and IFA group is performed in Table 4.

Table 4. Univariate analysis to compare the mean value of preconception nutrients intake between MMN group and IFA group using independent t test.

Nutrient	MMN Group n=57	IFA Group n=58	p value
Energy (cal)	1575 ± 375	1576 ± 478	0.996
Protein (g)	59.4 ± 17.7	59.8 ± 22	0.921
Fat (g)	65.5 ± 21.3	66.7 ± 27.7	0.801
Carbohidrate (g)	188 ± 59	181 ± 76	0.542
Dietary fiber (g)	8.2 ± 3.6	7.3 ± 3.7	0.179
Vit A (μg)	864 ± 885	845 ± 744	0.897
Thiamin (mg)	0.6 ± 0.2	0.6 ± 0.3	0.750
Ribovlavin (mg)	0.7 ± 0.3	0.6 ± 0.3	0.334
Piridoxine (mg)	0.9 ± 0.3	1.0 ± 0.4	0.866
Folic acid (μg)	118 ± 55	115 ± 63	0.828
Vitamin C (mg)	29.5 ± 30.4	22.6 ± 30.4	0.188
Iron (mg)	7.8 ± 2.9	7.6 ± 3.5	0.795
Zinc (mg)	7.3 ± 2.0	7.6 ± 3.0	0.571

Nutrient intake is also similar between treatment group and control group. There is no significant difference of preconception energy protein intake and other macronutrients intake such as carbohydrate, total fat and dietary fiber between two groups (table 4). The mean value of several micronutrients intake is also demonstrated similar result ($p > 0.05$).

Effect of Treatment on Maternal Hemoglobin Level

In this sub section, we start to discuss the effect of supplementation on maternal nutrition, and compare it between MMN group and IFA group. Treatment group received MMN during preconception period then continue during pregnancy (preconceptional MMN group) and control group received placebo during preconception period then continue with iron folic acid (IFA) during pregnancy (placebo-IFA group). Time series data of selected variables were performed in table 5.

A sequence of observation had been done in week-12, week-28 and week-35/36 to assess several data of subjects within MMN group and IFA group. The average of body weight in treatment group is increase from 47.42 kg before conception to 56.99 kg in week 35 -36, and provide weight gain of 9,57 kg in last trimester. Weight gain of subjects in MMN group is slightly higher compare to control group. The average of body weight in IFA group is increase from 47.26 kg before conception to 56.40 kg in week 35-36, weight of 9.14 kg.

Table 5. Times series data of subjects within MMN group and IFA group

Variables	Preconception n=57	Week-12 n=56	Week-28 n=56	Week-35/36 n=54	d
MMN Group					
Body weight (kg)	47.4 ± 8.1	49.3 ± 8.3	52.9 ± 7.9	57.0 ± 8.2	+ 9.6
MUAC (cm)	25.2 ± 3.3	25.4 ± 3.2	25.9 ± 3.1	26.2 ± 3.3	+ 1
Systole (mmHg)	106.2 ± 8.9	112.5 ± 5.5	110.7 ± 6.6	111.7 ± 6.1	+ 5.5
Diastole (mmHg)	70.7 ± 8.7	74.1 ± 5.9	75.2 ± 7.6	73.9 ± 5.9	+ 3.2
Hb level (g/dL)	12.2 ± 1.1	12.1 ± 0.7	11.8 ± 0.6	11.9 ± 0.3	- 0.3
IFA Group					
Body weight (kg)	47.3 ± 9.2	49.0 ± 8.9	53.6 ± 8.9	56.4 ± 0.1	+ 9.1
MUAC (cm)	25.3 ± 3.8	25.4 ± 3.5	26.1 ± 3.59	26.3 ± 3.2	+ 1
Systole (mmHg)	106.2 ± 9.9	112.9 ± 6.6	110.7 ± 7.1	112.3 ± 7.9	+ 6.1
Diastole (mmHg)	70.1 ± 8.4	76.4 ± 6.2	76.6 ± 5.9	75.5 ± 5.8	+ 5.4
Hb level (g/dL)	12.6 ± 1.2	12.3 ± 0.8	11.9 ± 0.8	11.9 ± 0.8	- 0.7

Effect of intervention on hemoglobin level is demonstrated in figure 2. Average of hemoglobin concentration tends to decrease in both groups. The initial Hb level of subjects within IFA group (control group) is higher compare to those in MMN group (treatment group), with the mean difference of 0.4 g/dL, then decrease in week-12 with mean difference of 0.2 g/dL, continue decrease until week-28, with mean difference of 0.1 g/dL.

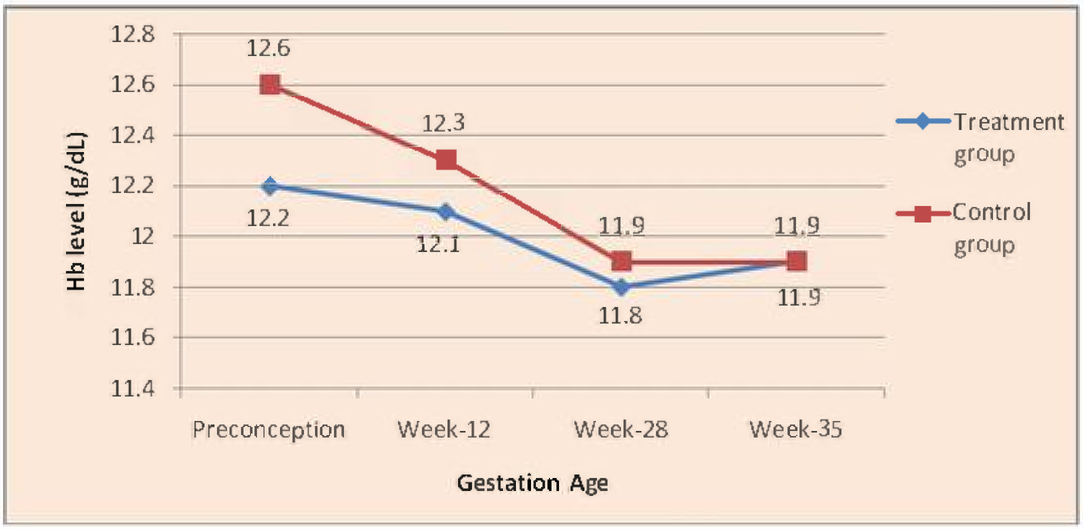

Figure 2. Average of hemoglobin concentration along gestation age between treatment (MMN) group and control (IFA) group.

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Table 6. Statistical analysis using independent t test for hypothetical testing of effect of treatment on hemoglobin level at baseline, week-12, week-28 and week-36 between MMN group and IFA group.

Parameter	t	df	Mean difference	Sig.(two tailed)
Hb level Baseline	-1.601	113	-0.337	0.112
Hb level in week-12	-1.315	109	-0.195	0.191
Hb level in week-28	-0.447	107	-0.062	0.655
Hb level in week-28	-0.171	97	-0.026	0.865

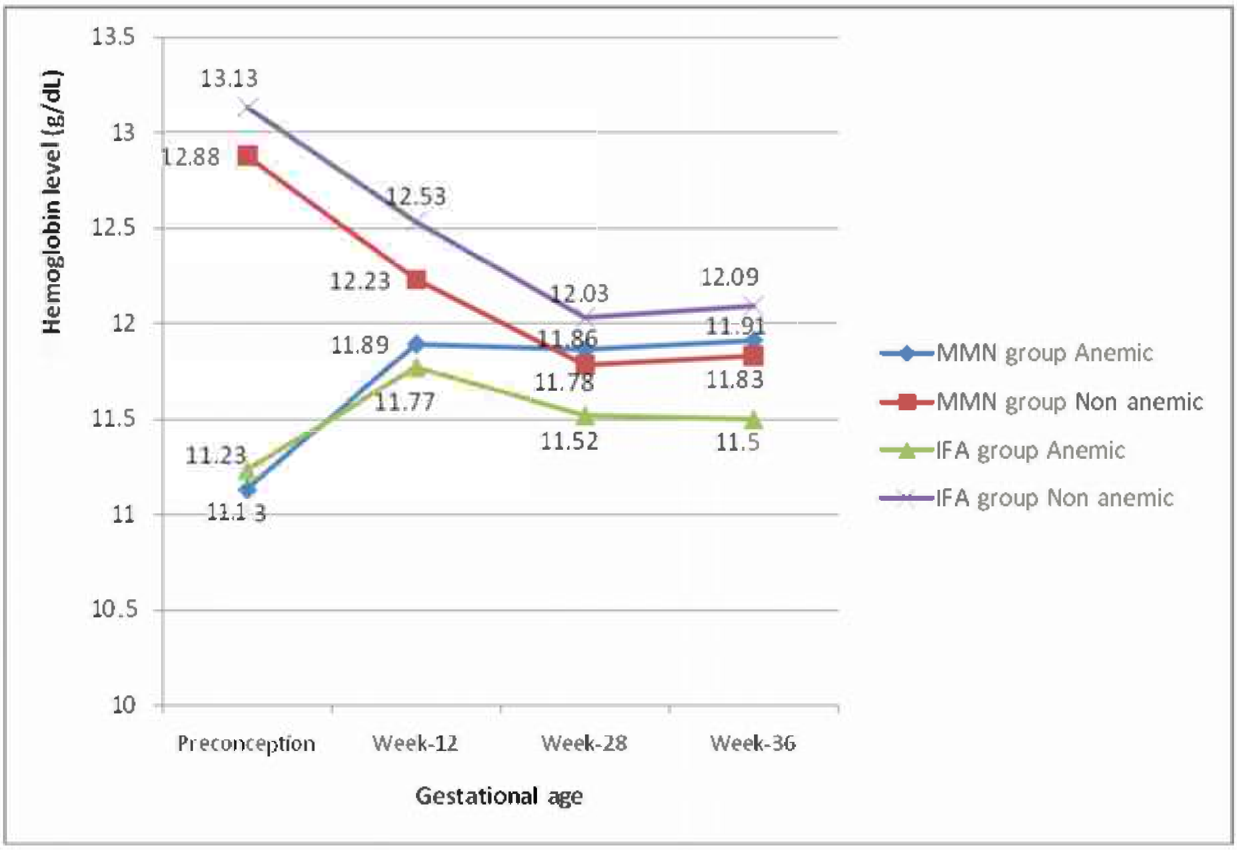

Figure 3. Average of hemoglobin concentration along gestation age among anemic and non anemic subject within MMN group and IFA group.

Base on statistical analysis, there were no significant different hemoglobin level in week 12 (p=0.191), week 28 (p=0,655), and week 35 (p=0,865) between MMN group and IFA group. The average of hemoglobin level at last pregnancy (week 35) more than 11 g/dL in both groups (table 6). Figur 3 shows the comparation of Hb level among anemic subjects and non anemic subject in in MMN group and IFA group.

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DISCUSSION

Statistical analysis using independet t test demonstrated that there is no significant differences of each variable between two groups. The mean value of most variables such as preconception body mass index and several body size including body weight, height, mid upper arms circumference, as well as hemoglobin level and blood pressure are comparable between subjects within treatment and control group ($p > 0.05$), unless age ($p=0.026$) is significantly different between two groups.

The average of energy intake in both group was less than dietary recommendation for Indonesian reference population, especially for women 16-29 years age (1900–2200 kcal/day). Energy intake among subjects in two groups is only reach of 70%-80% of the Indonesian recommended dietary allowences (RDA). Protein intake was adequate to meet their body requirement, because the average of protein consumption among subjects in treatment and control groups was 59.4 g and 59.8 g, respectively. This intake is higher than protein recommendation for these reference group (50 g/day). Most of micronutrients intake were less than dietary recommendation. Base on statistical evidence, it indicates that no significant differences of preconception characteristic of subjects between treatment group and control group. Therefore, it can be concluded that the two groups are comparable. We expect that any differences on several prospective outcomes it would be effect of the intervention.

From figure 2, we can see that the decreasing pattern of hemoglobin concetration is very interesting, because the mean difference between two group is tend to smaller according to gestation age, and finally no difference Hb level in week-35. These findings indicate that compare to iron folate, MMN supplementation provide efective elevation of Hb level particularly in last trimester. The decrease of hemoglobin concentration is follow the pattern of expansion in blood volume, but negatively corelated. In normal pregnancy, progressive decrease of hemoglobin concentration in second trimester (week 12-week 28), as physiological effect of progressive increase in plasma volume, and has usually reached a plateau by week 28 (Carriaga *et al.*, 1991). Our data demonstrate that Hb concentartion in control group is similar with Carriaga findings, that Hb level reached a plateau by week-28 and after. But in our treatment group, Hb level tends to increase by week-28. This elevation must be as the effect of MMN supplementaion.

When we focus in effect of treatment within group or before and after treatment, our result demonstrated a different evidence with the finding from study at West Java. This study revealed that at near term pregnant women who received weekly vitamin A and iron had

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significantly higher hemoglobin concentration compared with baseline. The hemoglobin concentration of anemic subjects (hemoglobin < 11 g/dL) increased from baseline by 1.07 g/dL in the weekly vitamin A and iron group, by 0.66 g/dL in the weekly iron group ($p< 0.01$) and by 0.34 g/dL in the daily group ($p< 0.05$). The increase of hemoglobin concentration in anemic subjects in the weekly vitamin A and iron group was significantly higher than in the daily group ($p< 0.05$). The hemoglobin concentration in nonanemic subjects did not change, and decreased from baseline by 0.41 g/dL in the daily group (Muslimatun *et al.* (2001). Our data more likely support a part of this finding. On the basis of initial hemoglobin concentration of our subjects at preconception, it was categorized non anemic (>12 g/dL), thus hemoglobin did not increase but decrease from baseline by 0.48 g/dL in both groups.

Figur 3 performs the interesting evidence that supplementation only works for anemic subjects, both of MMN and also IFA. During preconception period until week-12 of pregnancy, hemoglobin level of the anemic subjects increases progressively in both groups. Among anemic subject in MMN group, at week-12 there is increasing hemoglobin level of 0,76 g/dL from baseline, and increasing of 0.54 g/dL among IFA group. The interesting evidence is hemoglobin level among anemic subjects in IFA group after week-12 decrease until week-28 and then get plateau. On the other hand hemoglobin level among anemic subjects in MMN group after week-12 get plateau until week-28 and the slightly increase at week-36.

Base on statistical result in table 6, it implies that the eficacy of MMN preconception on iron status as efective as iron folic acid (IFA). More over, we have more detailed explanation descriptively, that in last terimester when hemoglobin concentration generally reached plateau in control group who recieved IFA, meanwhile subjects in treatment group who recieved MMN have increasing hemoglobin. It mean that process of erythropeisis during last trimester more efective in subject who received MMN. This condition more likely due to increasing iron mobilization which indicated by greater elevation of sTfR in treatmeant group rather than in control group. The increasing of iron mobilization might due to better vitamin A status among subjects within treatment group.

CONCLUSION

Prolonging supplementation of MMN during preconception will protect anemia or hemoglobin level less than 11 g/dL as affective as IFA supplementation during pregnancy which are represented by hemoglobin level at week-12, week-28 and week-36 of pregnancy. Both of intervention, whether supplementation of MMN or IFA, only works to increase hemoglobin level

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for the anemic subject before pregnancy, but it still can not increase the hemoglobin level for non anemic women before pregnancy.

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