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The Effect of Selenium and Multiple Micronutrient Administration during Periconceptional Periodon Selenium Status, Malondialdehyde (MDA), sFlt1, and PIGF to Prevent Preeclampsia at the Molecular Level

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Abstract

The role of Selenium in preventing preeclampsia has been proved in some studies. It is evident that the decrease of Selenium level results in oxidative stress in the placenta, leading to preeclampsia symptoms. The relationship between Se status and preeclampsia incidence is shown in some studies in 45 countries. This study aims to prove the effect of Selenium on women during the period of periconception to Selenium level in plasma, MDA, Sflt-1, PlGf, to prevent preeclampsia incidence at the molecular level. The research use the double blind, randomized, control trial design. The samples are women in praconceptional period (34 samples in each group). The intervention group will receive Selenium in MMN preparation, while the control group will receive capsules containing ferrum and folic acid (national program).

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During praconceptional period, the capsules will be given once a week, while pregnant women will be given the capsules once a day. The levels of selenium and MDA will be examined before the treatment. At the 12th and 20th weeks of pregnancy, selenium, MDA, Sflt-1,and PlGF were measured.) Selenium and MDA levels will be measured statistically using the t-test pre-post and paired t-test, while the Sflt-1/PlGF ratio will be determined using a t-test to compare the two groups. Early prevention of preeclampsia can be conducted by observing preeclampsia markers before the occurence of clinical symptoms and signs. It is expected that this study can contribute to the prevention of preeclampsia through nutrition intervention since the praconception period.

Keywords: Periconception; Selenium; MDA; Sflt-1; Preeclampsia.

1. Introduction

Until now preeclampsia is still the main cause of mortility and morbidity among mothers and their babies[1]. In Indonesia, preeclampsia is the second most frequent cause of mother mortality [2]. Although mother death rate is lower in high-income countries, 16% of mother mortality cases are due to preeclampsia [3].

The cause of preeclampsia has not been exactly identified, but the underlying causes of the incidence are trophoblast invasion and remodelling problems. One of the molecular level mechanisms contributing to pathogenic preeclampsia is the Altered Angiogenic Balance [4]. The mechanism of micro nutrient role in preventing preeclampsia has not been completely identified, but it can be explained through antioxidant and oxidative stress, as nutrition can balance and prevent free radicals which cause oxidative stress during pregnancy [5].

Efforts to preventpreeclampsia have been done through intervention using antioxidant micronutrient, because there is an evidence of the involvement of placental oxidase [6]. Micronutrient substances are strongly correlated with complications during pregnancy such as preeclampsia. Oxidative stress plays a role in such incidence. In some journals, it is claimed that the intervention is not effective, and there is even a potentiality to be harmful [7]. Therefore, an intervention is started with the use of Selenium. Selenium is an essential micromineral component of Glutathione peroxidaseenzym. This enzym, together with superoxide dismutase (SOD) catalystand vitamin E have a strong antioxidant power to survive oxidative damage by free radicals [8]. Selenium is a part of antioxidant enzym that protects cells and lipid membrane from oxidative damage [9].

The role of Selenium in preventing preeclampsia has been proved in some researches [10,11]. It is evident that the decrease of Selenium level results in oxidative stress in the placenta, leading to preeclampsia symptoms. The relationship between Se status and preeclampsia incidence is shown in some researches in 45 countries. Se supplementation among women in China can prevent hypertension and oedema during pregnancy. The level of Se in serum/plasma and GPx is significantly lower in pregnancy with preeclampsia than in normal pregnancy [6] while low levels of selenoenzym GPx, and thioredoxin reductaseare mostly found in preeclampsia than in normal women [12]. An interesting study on animals tries to show that diet without Se results in some symptoms like preeclampsia, including the increase of blood pressure, proteinuria, and oxidative stress in the placenta compared to the use of normal Se [12,13].

The role of selenium intervention in preventing preeclampsia is related to sFlt-1 level. A study by Rayman, M.P [11] shows that at the 12th and 35th weeks of pregnancy, selenium concentration increases in pregnant mothers receiving selenium supplement intervention, but decreases in the control group. So far, the research on the influence of Se intervention during pregnancy on sFlt-1 is conducted by administeringselenium only Rayman MP [11].

The writers have not found any research on selenium intervention withMultiple Micronutrientpreparation. It is expected that the combination of selenium and other antioxidant such as vitamin A, C, and E, and antioxidant minerals such as ferrum, zinc, and copper can provide a synergic effect against free radicals, prevent placental oxidative stress, and decreaese sFlt-1 antiangiogenic, leading to the prevention of endothelium tissue that can increase the risk of molecular preeclampsia. It is then necessary to conduct a research about the effect of Selenium intervention in (Multiple Micronutrient/MMN)preparation during periconception period on the levels of selenium, malondialdehyde (MDA), sFlt-1,and PIGF to prevent preeclampsia at a molecular level. This research aimed to to assess the effect of giving Multiple Micronutrient supplement containing selenium during the perconception period on the selenium level, malondialdehyde (MDA), and sFlt-1/PIGF ratio in pregnant mothers to prevent preeclampsia at the molecular level.

Objectives

1. To analyse the level of selenium before and after the administration of Multiple Micronutrient supplement containing selenium in the intervention and control groups.
2. To analyse the level of malondialdehyde (MDA) before and after the administration of Multiple Micronutrient supplement containing selenium in the intervention and control groups.
3. To analyse the level of MDA at the 12th and 20th weeks of pregnancy in the intervention and control groups.
4. To analyse the ratio of sFlt-1/PlGF at the 12th and 20th weeks of pregnancy in the intervention and control groups.

Hypothesis

1. The level of Selenium of pregnant mothers receiving Multiple Micronutrient supplement containing selenium is higher than the one of the group of pregnant mothers receiving Fe+folic acid.
2. The level of malondialdehyde (MDA) at the 12th and 20th weeks of pregnancy is lower in pregnant mothers receiving Multiple Micronutrient supplement containing selenium than in the group of pregnant mothers receiving Fe+folic acid.
3. The ratio of sFlt-1/PlGF at the 12th and 20th weeks of pregnancy is lower in pregnant mothers receiving Multiple Micronutrient supplement containing selenium than in the group of pregnant mothers receiving Fe+folic acid.

2. Materials and Methods

2.1 Study design and intervention

The research will be conducted in three subdistricts in Banggai regency namely Luwuk, South Luwuk, and North Luwuk. These three subdistricts are selected purposively as the representation of Banggai regency area based on some considerations. First, the rate of preeclampsia is high in these three areas. Second, the three locations are accessible, making it possible for monitoring and controlling the intervention. Third, the three subdistricts are similar in terms of the socioeconomic aspect. Finally, people mobility in the three subdistricts is relatively low.

This study can be considered a true experimental research using the double blind community-based trial design. It can also be regarded as an efficacy study aimed to analyse the advantage or benefit of an intervention under a tight control .

The scheme description is as follows:

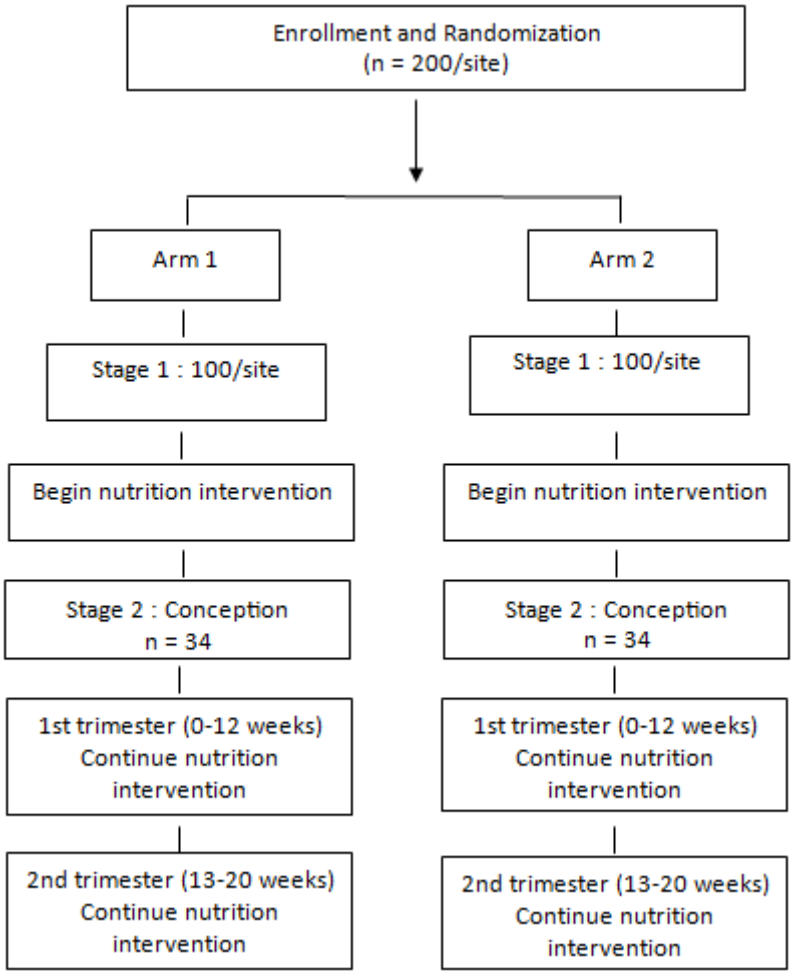

Figure 1: Consort diagram (subject numbers are for each independent site).

2.2 Sample Size Calculations

The number of samples is determined with a 90% power to detect 386 differences of sFlt-1 concentration, with type I error in $\alpha = 0.05$ on one side, standard deviation (SD) 772 (based on the study of Mousavi M, [14]. There

are 34 periconcenptioal mothers in each group with an anticipation value DO 20% and design effect [15].

Based on the calculation above, 34 samples are needed in each group until the end of the observation. Considering that the percentage of newly married women who get pregnant less than six months is 50%, and the median value of time to pregnancy is about six months (Otoluwa, 2014), it is necessary to have samples of 100/50X6=68. The predicted drop out and handling lab error is 20% (d=0.2), so that the number of samples is multiplied with the factor of 1/(1-d). The multiplier factor is 1/1(1-0.2) = 12.5, so that the number of samples becomes 68 x 1.25 = 85. Based on the calculation, the study needs to involve 85 samples in each group, so that the total number of the samples needed in the recruitment is 2 x 85 = 170preconceptional mothers.

2.3 Subject Recruitment, Randomization to Treatment

The research population includes preconceptional mothers who register in the program of Praconception Integrated Service Centre. They are from three subdistricts in Banggai regency. The samples are eligible respondents based on some inclusion criteria: praconceptional mothers, do not have previous pregnancy, married, aged 18-35 years old, plan to be pregnant in less than one year, and agree to participate in the research by signing an informed consent. The exclusion criteria are preconceptional mothers with hyperglycemia (DM),kidney failure, hypertension, and tuberculosis; not a permanent citizen in the areas (stay duration < 6 months), not living with husband, twin pregnancy, obesity (IMT>30), severe anemia (<7g/L), upper arm circumference < 23.5 cm, positive proteinuria (++), six-month participation in the intervention program but not getting pregnant, have been married > 6 years but never get pregnant.

A block IV randomisation results in a group receiving selenium and Multiple Micronutrient, and a control group receiving program capsules containing ferrum and folic acid. The MMN capsules containing Se and other Multiple Micronutrient will be given once a week when the mothers are not pregnant, and once a day during pregnancy. The intervention will be stopped when after six-month intervention, the mothers do not get pregnant so that the respondents will be considered dropped out.

2.4 Pre-pregnancy supplement distribution, measurement of compliance and detection of pregnancy

The distribution of intervention and control capsules to mothers will be conducted starting from the researcher to subdistrict coordinators, who will then distribute the capsules to village midwives. These village health workers will distribute the capsules among the cadres of integrated service centres in each village. Respondents will be visited once in a week to distribute the MMN capsules as well as to control the adeherence in consuming the MMN capsules. During the research, the mothers will not be allowed to consume vitamin and minerals from outher sources. Any violation of this requirement will result in the cancellation of respondents' participation in the study.

2.5 Data collection

Types and time of data collection can be seen in the following table.

Table 1: The composition of iron folic acid and multiple micronutrient supplements

Ingredient	Preconception (weekly)		RDA for non-pregnant women
	IFA	MMN	
Vitamin A, RE		800	700
Vitamin B12, µg		2,6	2,4
Vitamin B6, mg		1,9	1,3
Riboflavin (B2), mg		1,4	15
Vitamin C, mg		70	75
Vitamin D, IU		10	15
Vitamin E, mg		10	15
Zinc, mg		15	8
Iron, mg	600	30	18
Niacin, mg		18	14
Selenium µg		65	55
Copper, mg		2	900 mcg/day
Folate, µg	400	400	400
Iodine, µg		150	150

2.6 Pregnancy supplementation, monitoring and follow-up during pregnancy

Pregnant mothers will be required to consume one supplement capsule before night sleeping every day. The cadre will distribute the supplements every fortnight, and at the same time, the cadre will monitor the the consumption. In addition, the cadre will also record side effects and any pain felt by the mothers. Furthermore, the pregnant mothers will have blood pressure examination, proteinuria assessment, and anthropometric measurement. Their blood samples will also be taken to determine the levels of hb, glucose, selenium, and MDA at the first stage. At the 12^{th} and 20^{th} weeks of pregnancy, blood sample examination will be conducted to measure selenium level, MDA, and the ratio of sFlt-1/ PIGF at the second stage. The pregnant mothers will also

have blood pressure measurement every month, and proteinuria examination at the 20th week of pregnancy. After the samples are taken for examination, the administration of Multiple Micronutrient will be continued until the time close to baby delivery. The examination of Se level, MDA, and the ratio of sFlt-1/PIGF and ET-1 will be conducted in the Clinical Pathology Laboratory of Dr. Soetomo hospital. The pregnancy examination will be conducted by village midwives at the Intergated Service Centre or Village Health Centre. Pregnant mothers experiencing complications will be referred to an Obstetrics and Gynecologyspecialist [16,17].

Table 2: Data collection and measurements

Measurements	Prekonsepsi	Pregnancy	
		12 weeks	20 weeks
Demographics, SES status	√		
OBGYN history	√		
Environment sanitation	√		
Exposure to pollutans	√		
Access to health and nutrition information	√		
Morbidity	√		
Hematological measures	√	√	√
Anthropometry	√	√	√
Food Recall 24 Hour	√	√	√
Food frequency questionnaire	√	√	√
Hemoglobin	√	√	√
Blood pressure	√	√	√
Urine Protein	√	√	√
Urine Glucose	√	√	√
Urin PH	√	√	√
Blood Selenium	√	√	√
Blood MDA	√	√	√
Blood sFlt-1		√	√
Blood PlGF		√	√
Supplement consumption	√	√	√
Side effects	√	√	√
		√	√

2.7 Follow-up with women who do not become pregnant

Mothers who do not become pregnant until 6-month administration will be excluded from this study. However,

when they quit the research,their Hb level will be examined.

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