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The Effect Of Multi-Micronutrient Supplementation Since Preconception On Levels Of Malondialdehyde (Mda) For Pregnant Women

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ABSTRACT

The nutritional status of a mother before and during pregnancy is important for a healthy pregnancy outcome. The preconception period is a critical time for improving the nutritional status of pregnant women and preventing pregnancy complications. (MMN) compared to iron folate (IFA). Methodology: A double-blind randomized cohort study was carried out in three districts of Luwuk, South Luwuk, and North Luwuk, Banggai Regency with a total sample of 19 pregnant women given MMN supplementation and IFA supplementation. See the effect of multi-micronutrient supplementation during pregnancy on MDA levels measured several times (preconception, trimester 1, trimester 2, trimester 3). Results: MMN supplementation group MDA levels from preconception (144.43) decreased in trimester 1 (127.60) to trimester 2 (130.94) again until trimester 3 (96.03), while the average IFA supplementation group MDA from preconception (110.54), increased in trimester 1 (153.87) increased again in trimester 2 (172.50) then decreased in trimester 3 (81.75), a decrease in MDA levels was better in the MMN supplementation group.

Keywords: Pregnancy, Preconception Period, Malondialdehyde, Supplementation.

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INTRODUCTION

Malnutrition before and during pregnancy can cause adverse perinatal results¹. Nutritional deficiencies in pregnant women can cause maternal deaths associated with iron deficiency anemia due to bleeding after childbirth (SDKI, 2012). Malnutrition in pregnant women can interfere with fetal development and contribute to the low birth weight which results in stunting (UNICEF, 2013). Nutritional deficiencies during pregnancy permanently cause non-communicable diseases such as coronary heart disease, cancer, and diabetes².

Micronutrient deficiencies in reproductive women and pregnant women, according to Cochrane review and the Lancet series recommend the use of iron, folic acid, and multi micronutrient supplementation to reduce anemia and low birth weight as a form of specific interventions³. Micronutrients are vitamins and minerals that are needed for the body's normal functioning, growth, and development. Mothers in low-income countries often consume inadequate micronutrients due to limited intake of animal products, fruits, and vegetables. Lack of micronutrients in pregnancy can worsen conditions that lead to potential side effects in the mother such as anemia and even death⁴. Mineral and vitamin deficiencies have no clinical symptoms; can damage intellectual development, cause illness and death of pregnant women, and weak immune system. (Unicef, 2013).

Preconception nutritional status plays an important role in optimizing pregnancy outcomes, maternal health and producing a good generation. Maternal nutrition affects fetal and maternal perceptual nutrition is needed for long-term children's health. Suboptimal outcomes are related to maternal preconception nutrition for example stunting⁵.

Reactive Oxygen Species (ROS) increase due to the absence of antioxidants such as vitamin A, vitamin C and vitamin E⁶. ROS, for example hydroxyl radicals, hydrogen peroxide, and oxygen *singlets*, result from aerobic metabolism and *phagocytic* processes. During respiration in the mitochondria, producing superoxide radicals, converted to hydrogen peroxide by superoxide dismutase producing hydroxyl radicals can cause fat *peroxidation*, with the end produce of *malondialdehyde* (MDA) *genotoxic* and *mutagenic*⁷.

Lipids are an essential component of cell membranes that maintain cell structure and function. Lipids are the main target of ROS attacks such as oxygen free radicals and lipid oxidation is associated with various pathologies. Polar lipids are components of the structure of cell membranes, participating in the formation of cell barriers. Most of these are *lipid bilayers*, in almost all membranes they are lipids based on glycerol. Lipid membranes are important in controlling the physiological state of the membrane by modifying its biophysical aspects, such as polarity and permeability⁸.

Vitamin C (*ascorbic acid*) plays a role in removing unrestricted radicals and inhibiting lipid **peroxidation**. Vitamin E and vitamin C are antioxidants to protect against biological membrane damage by their ability to eliminate free radicals⁹. Based on the above background it is necessary to research *multimicronutrient* supplementation and IFA with markers of membrane damage (MDA) for pregnant women.

MATERIAL AND METHODS

This study gave multi-micronutrient tablets to mothers from preconception to delivery, from September 2016 to

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January 2018, as many as 19 preconception mothers, followed by pregnancy until delivery and examination of membrane damage (MDA) every trimester. The study was carried out in 3 districts namely Luwuk, North Luwuk and South Luwuk, in Banggai Regency, Indonesia. A prospective cohort study design with a double-blind randomized controlled trial study design.

The Content of Multi Micronutrient Capsules

Multi-micronutrient capsules from UNICEF are produced by Loma Pharm Germany, which contains Se 65 mcg, Retinol (vitamin A) 800 ug, Vitamin E 10 IU, Vitamin D 200 IU, Vitamin B1 1.4 mg, Vitamin B2 1.4 mg, Vitamin B2 18 mg, Vitamin B6 1.9 mg, Vitamin B12 2.6 mcg, Folic Acid 400 mcg, Vitamin C 70 mg, Iron 30 mg (iron sulfate), zinc 15 mg (zinc sulfate), copper (Copper) 2 mg, Iodine 150 micrograms. Given during the preconception as much as 1 time a week and once a day during menstruation and for the period of pregnancy are given every day during pregnancy.

The Content of Iron Capsules + Folic Acid

A capsule containing 60 mg Fe and 400 micrograms of folic acid is given at the time of preconception as much as 1 time a week and 1 time a day during menstruation and for pregnancy given every day during pregnancy.

BIOCHEMICAL METHODS

Blood is collected in the morning after overnight fasting, using a Vacutainer blood collection tube and transported to the local laboratory location for processing within 1 hour after blood is drawn. Blood is immediately processed for the separation of plasma and red blood cells by centrifugation at 2000 rpm for 10 minutes. Plasma samples are stored at 80°C then transported to the Laboratory for MDA measurement. Measuring MDA concentration is a lipid *peroxidation* product, MDA measurements are determined using commercially available sandwich-ELISA kit (Elabscience, Wuhan, China), adduct obtained with *thiobarbituric acid* (TBA).

Measuring Compliance

The research team and midwives at *poskesdes* (village health posts) or *puskesmas* (community health centers) visit Women have to offer supplements every two weeks, monitor obedience, and record signs or side effects that women may have. Researchers and midwives directly saw the consumption of one tablet every day. Respondents are given a monitoring and evaluation sheet filled for 1 week by the respondent. Every week respondents are required to collect monitoring and evaluation sheets to researchers and or midwives at *poskesdes/pustu/puskesmas*. Researchers have WhatsApp groups and *arisan* (regular Social Gathering) groups. The WhatsApp group to facilitate communication between the research team and the respondent also accommodates all questions and complaints related to the research that respondents might feel. Twice a month the research team and respondents held a meeting in the form of social gathering, as well as educating and answering questions about reproductive health and nutrition 1000 hpk. The research team and midwives called or used text messages between visits to remind participants to take the next pill. Compliance is based on sheets submitted to researchers and midwives counting and monitoring and evaluating empty capsule packages stored in women's homes. Women are encouraged to take supplements on an empty stomach at the same time every week to reduce compliance, absorption, and side effects.

RESULT

Malondialdehyde (MDA), the most popular indicator for oxidative damage to cells and tissues, can be used as a marker of cell membrane damage. In pregnancy, the rate of cell division occurs not only in the fetus, placenta but also in the maternal compartment (red blood cells and uterine growth). Additional antioxidants are needed to fight the oxidative stress that occurs during pregnancy. Excess oxidative stress will attack endothelial cells in blood vessel walls. Oxidative stress will also attack lipids, proteins, carbohydrates, and DNA¹⁰.

Table 1: Comparison of MDA levels in preconception, trimester 1, trimester 2 and trimester 3 Iron folic acid (IFA) and multi micronutrient (MMN) groups.

MDA	IFA supplementation group			MMN supplementation group			p ¹
	Mean ± SD	Media n	Min -max	Mean ± SD	n	Min -Max	
Preconception n	110.54 ± 80.82	63.20	54.5 - 274.7	144.43 ± 91.27	138.15	41.00 - 382.40	0.31
Trimester 1	153.87 ± 63.27	130.20	82.3 - 245.3	127.60 ± 74.78	116.40	54.30 - 330.40	0.35
Trimester 2	172.50 ± 93.53	120.60	93.4 - 349.2	130.94 ± 85.20	105.55	27.20 - 350.00	0.20
Trimester 3	81.75 ± 30.08	92.40	38.7 - 121.9	96.03 ± 31.03	89.05	52.80 - 170.20	0.55

¹ Mann Whitney Test

In the Table 1 shows an average increase in MDA levels from preconception (110.54), trimester 1 (153.87) to trimester 2 (172.50) and then decrease in trimester 3 (81.75) of the IFA supplementation group. While the

MMN supplementation group MDA levels from preconception (144.43) decreased in trimester 1 (127.60), until trimester 2 (130.94) decreased again until trimester 3 (96.03), a decrease in MDA levels was better in the MMN supplementation group.

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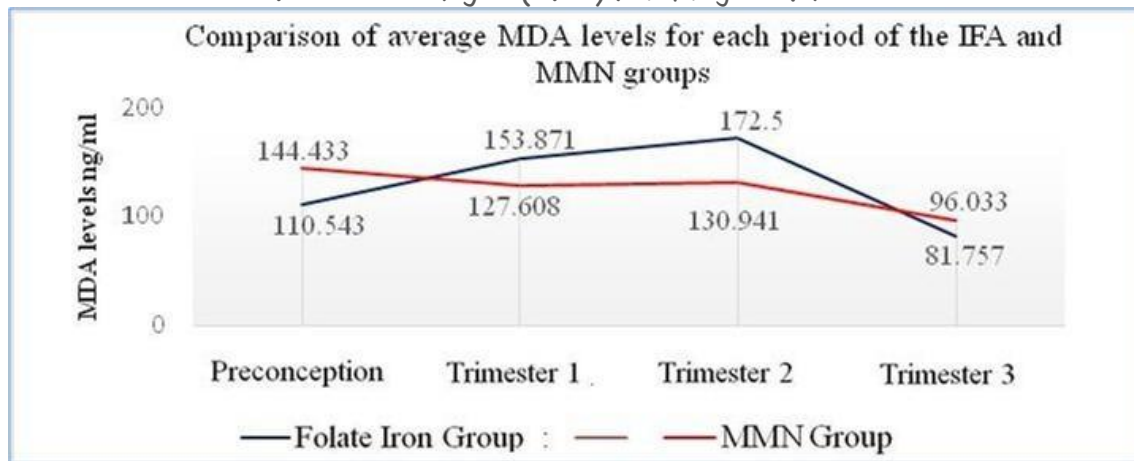


Fig.1: Comparison of average MDA levels for each period from preconception to trimester 3 between the IFA and MMN groups

In the Fig. 1, it can be seen that in the second trimester there is peak of MDA damage, MMN supplementation (130,941) can further reduce MDA levels compared to IFA supplementation (172.50).

DISCUSSION

In the table above shows the MDA levels of MMN supplementation group from preconception (144.43) decreased in trimester 1 (127.60) to trimester 2 (130.94) decreased again until trimester 3 (96.03), while the IFA supplementation group average MDA levels from preconception (110.54), increased in trimester 1 (153.87) increased again in trimester 2 (172.50) and then decreased in trimester 3 (81.75). In the table above shows the decrease in MDA levels from preconception to trimester 3, MMN supplementation reduced MDA levels greater (48.4) compared to IFA supplementation (28.78) although not significantly different ($p = 0.59$).

In multiple micronutrients containing vitamin B1, selenium, vitamin E, vitamin C, zinc, and copper may play a role in reducing MDA levels. Vitamin B1 (thiamine) is a cofactor for the formation of NADPH which plays a role in the synthesis of glutathione as an antioxidant¹¹. Porcelain component of the glutathione peroxidase enzyme¹², prevents the formation of lipid peroxidation through peroxid reduction (radicals) intermediates¹³. Vitamin E as the main antioxidant in the lipid phase acts as a scavenger of free radicals through non-enzymatic mechanisms. The role of vitamin E during the lipid peroxidation process will donate an electron to the peroxyl radical intermediates, ending the peroxidation reaction^{14,15}. Vitamin C, as a free radical scavenger, Vitamin C recycles α -tocophenoxyl radicals into a form of antioxidants⁹. Copper and zinc act as antioxidants, involved in the activity of antioxidant enzymes, specifically SOD¹⁶. Superoxide dismutase converts superoxide (O_2^-) to H_2O_2 , often called the main defense, because this enzyme prevents radical generation¹⁷.

During pregnancy hormonal changes occur resulting in accumulation of maternal fat which correlates with fetal lipids and fetal growth. The presence of phospholipid membranes at the site of ROS formation will be easily accessed as a lipid peroxidation target. MDA is one of the final products formed from the breakdown of primary and secondary lipid peroxidation products¹⁸. In trimester 1, the formation of fetoplasmic circulation requires the invasion of extravillous trophoblasts into the

maternal spiral arteries. At the age of 8 weeks of pregnancy, trophoblast blockage in a spiral artery protects the embryonic from oxidative stress and hypoxia in the placenta¹⁹. Environments with low oxygen levels produce ROS which not only have a direct effect on cells but is also active as second messengers for the regulation of transcription factors that alter gene expression in embryos. Hypoxia-inducible factor (HIF-1) regulates transcription genes related to energy metabolism such as erythropoiesis, angiogenesis. HIF-1 is activated during hypoxia, regulating vascular development through regulation of vascular endothelial growth factor (VEGF). NF- κ B transcription factors, cytokine regulators and antiapoptotic gene expression. Changes in NF- κ B are indicated by changes in cellular proliferation and apoptosis through the regulation of the BCL gene²⁰. Increased activity of antioxidant enzymes causes inactive ROS and a decrease in lipid peroxidation. A balance between the production of ROS and antioxidants will protect the tissue from damage. MDA is a metabolite of lipid peroxidation production detected in plasma and used as an indicator of lipid peroxidation. Catalase, superoxide dismutase (SOD) and glutathione peroxidase are components of the antioxidant defense system that controls free radicals in cells. lipids in trimester 3. In trimester 2 and trimester 3, there is an increase in glutathione peroxidase. Increased glutathione peroxidase activity is a form of fetal protection against the effects of hydrogen peroxidase. Increased glutathione peroxidase can reduce MDA levels in trimester 3²¹.

Research Chatziralli IP et al, 2017 states the provision of 300 mg of vitamin E every day for 3 months in diabetics can reduce levels of MDA.¹⁴ Vitamin C 100 mg, given daily during the 2nd trimester of normal pregnancy, can significantly reduce MDA levels. Giving vitamin C 500 mg for 2 months can reduce MDA, as well as giving vitamin E 400 IU for 2 months can reduce MDA⁷.

Research Basu J et al, 2015 states the highest levels of placental MDA in trimester 1 and then decreased in trimester 2 and trimester 3 in normal pregnancy.²² Hypoxia occurs in trimester 1 when the invasion of trophoblast extravilli in the spiral arteries clogs the maternal spiral arteries so that hypoxia occurs this condition cause's oxygen pressure in the placenta is lower than in the endometrium. ROS in early pregnancy can affect the remodeling of the spiral arteries and the proliferation of cytotrophoblast cells. as a determination of the pathological state in pregnancy.Oghagbon SE et al. 2016 study state pregnancy is a condition that requires high metabolism with high oxygen requirements, resulting in increased oxidative stress.²³ MDA levels

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increase progressively during pregnancy, increase in trimester 2 and trimester 3. Uncontrolled production of MDA due to an increase in oxidative stress can significantly impair cell integrity. An increase in SOD antioxidants in several studies but there are also antioxidants that may decrease in response to lipid peroxidation as a pathological form. In this study, an increase in SOD while the catalase and glutathione peroxidase levels decreased. A study from Bassi R et al., 2017 states that pregnancy is a state of physiological stress in the body and signals to respond by increasing the formation of antioxidants to protect tissues.²⁴ The accumulation of antioxidants in the placenta prevents the detrimental effects of superoxide. In this study MDA levels increased significantly in trimester 2 and trimester 3²⁷. An increase in MDA levels corresponds to an increase in pregnancy and correlates with an increase in lipid peroxidation. MDA is the final product of lipid peroxidation²⁶.

Hasan IS Research, Laylani LAS, 2017. An increase in MDA levels in trimester 2 and trimester 3 during normal pregnancy.²⁵ A slight increase in oxidative stress even though the levels of catalase, glutathione peroxides and vitamin C MDA are antioxidants such as increase with gestational age. Increased oxidative stress during pregnancy can harm the health of mother and fetus.

CONCLUSION

MMN supplementation group MDA levels from preconception (144.43) decreased in trimester 1 (127.60) to trimester 2 (130.94) again until trimester 3 (96.03), while the average IFA supplementation group MDA from preconception (110.54), increased in trimester 1 (153.87) increased again in trimester 2 (172.50) then decreased in trimester 3 (81.75), a decrease in MDA levels was better in the MMN supplementation group.

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