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Original Article

The Effect of Moringa Leaf Extract Intervention Since Preconception Period on the Prevention of Oxidative Stress in Pregnant Women and Adverse Pregnancy Outcomes

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ABSTRACT

Background: Oxidative stress occurs due to an imbalance of oxidants and anti-oxidants and is often associated with poor pregnancy outcomes. Therefore, additional supplementation is needed since the preconception period to prevent oxidative stress and poor pregnancy outcomes. This study aims to examine the effect of supplementing Moringa leaf extract on the prevention of oxidative stress in pregnant women and poor pregnancy outcomes. **Methods:** This study is an observational study with a prospective cohort study design. The research subjects were third trimester pregnant women consisting of 2 groups, namely the control group who only received Iron-Folic Acid (IFA) supplements (n = 30) and the group that received moringa leaf extract and IFA supplements (n = 26). Then an examination of Malondialdehyde (MDA) levels as a biomarker of oxidative stress was carried out using the ELISA (enzyme-link immune sorbent) method, followed by pregnancy outcomes including birth weight and birth length. Data were analyzed by unpaired t test. **Results:** The results showed that there were no significant differences in the levels of MDA, birth weight and birth length in the two groups with a p value > 0.05. Nevertheless, MDA level in IFA group was 49.25 nmol/ml higher than Moringa + IFA group which was 47.58 nmol/ml. Likewise, the average of birth weight and birth length in Moringa group were 3042.3 grams and 48.62 cm higher than IFA group which were 2993.7 grams and 48.23 cm. Malondialdehyde levels were also higher in women who had babies with birth weight < 2500 grams was 49.48 nmol/ml and birth length < 48 cm was 49.13 nmol/l compared to birth weight ≥ 2500 grams was 48.50 nmol/ml and birth length ≥ 48 cm was 48.62 nmol/ml but there was no significant difference in the two groups (p>0.05). **Conclusion:** Oxidative stress in pregnant women tends to be associated with poor pregnancy outcomes. Moringa leaf extract supplementation since preconception can prevent increased oxidative stress and improve pregnancy outcomes.

Key words: Oxidative stress, Malondialdehyde, *Moringa oleifera*, Pregnancy outcomes.

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BACKGROUND

Oxidative stress is a condition that occurs due to an imbalance between oxidants and anti-oxidants. Oxidative stress is the result of excessive free radical production and failure of the anti-oxidant defense mechanism to produce a product called reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can interfere with signaling and control mechanisms and cause damage to molecular level.[1,2]

An increase in metabolic activity and a decrease in anti-oxidative activity in pregnant women causes excessive oxidative stress which results in complications in pregnancy outcomes including pre-eclampsia, hypertension due to pregnancy and gestational diabetes mellitus, abortion, intra uterine growth restriction (IUGR), premature birth, birth death, low birth weight babies (LBW) and stunting, all of which can increase perinatal mortality.[3] Oxidative stress shown by increased PrCarb (Protein Carbonyl) is associated with low birth weight and head circumference as well as small gestational age.[1]

Several ways have been carried out to improve poor pregnancy outcomes such as LBW and stunting, including by increasing education for pregnant women and their families through ANC (Antenatal care) services.[4] In addition, currently being encouraged to provide adequate nutrition since the pre-conception period. The preconception period is the time span from three months to one year before conception. The preconception period is an important period for optimizing pregnancy outcomes.[5,6] Several nutrients can affect pregnancy outcome by affecting maternal and fetal metabolism through their role in modulating oxidative stress, enzyme function, transduction pathways and signal transcription that occur early in pregnancy.[7,8]

Moringa is a herbal plant that has a complete nutritional content, so it is widely used as food and alternative medicine throughout the world.[9] Based on research, moringa contains a variety of essential nutrients, for example, vitamins, minerals, amino acids, β-carotene, antioxidant, anti-inflammatory, omega 3, omega 6 and essential fatty acids.[10] Moringa leaves are believed to be a high source of vitamin C, calcium, β-carotene, potassium and protein. Moringa leaves are an effective source of natural antioxidants because they contain several antioxidants including flavonoids, ascorbic acid, carotenoids and polyphenols.[11] Moringa is reported to contain vitamin C 7 times stronger than oranges, vitamin A 10 times that of carrots, calcium 17 times

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that of milk, protein 9 times that of yogurt, potassium 15 times that of bananas and iron 25 times that of spinach, polyphenols 2 times that of red wine. and GABA (gamma-aminobutyric acid) 100 times that of brown rice.[12]

Several studies have proven the benefits of giving moringa to pregnant women and pregnancy outcomes. Giving Moringa leaf extract to pregnant women 12 weeks during pregnancy and 4 weeks after giving birth can prevent malnutrition and reduce morbidity in infants aged 0-5 months.[13] Giving Moringa leaf extract to pregnant women can prevent anemia by increasing serum ferritin and preventing LBW.[14] In addition to improving pregnancy outcomes, Moringa leaves can also prevent oxidative stress in pregnant women. Giving Moringa leaf extract to pregnant women informal workers can reduce stress and increase upper arm circumference.[15] In another study, the results were the same where there was a decrease in MDA levels after the intervention of moringa leaf extract and royal jelly.[16] Therefore, we wanted to further examine how the relationship between oxidative stress in pregnant women and pregnancy outcomes and to see the effect of giving Moringa leaf extract since the preconception period on pregnancy outcomes through its effect on preventing oxidative stress.

MATERIAL AND METHODS

This research is a prospective cohort study and was conducted in North Polongbangkeng District, Takalar Regency. Polongbangkeng sub-district consists of 9 sub-districts and North Polongbangkeng sub-district consists of 6 sub-districts and 12 villages. This research was conducted from 2021 to 2022. The research subjects were third-trimester pregnant women. Group 1 are pregnant women who receive IFA supplementation as much as one capsulse per week during preconception and one capsule per day during pregnancy or at least 90 capsules and group 2 are pregnant women who receive Moringa leaf extract + IFA supplementation as much as one capsule per week during preconception and one capsule per day during pregnancy or at least 90 capsules. In one IFA tablet contains 60 mg Fe + 0.40 mg of folic acid, one Moringa capsule contains 600 mg of Moringa leaf extract.

Data was collected through a questionnaire containing information about the respondent's age, mother's occupation, mother's education, father's occupation, father's education, family income, oxidative stress questionnaire and food recall within 24 hours. Malondialdehyde measurements were carried out in the Hasanuddin University Teaching Hospital laboratory. 5 ml of the respondent's whole blood was collected and then centrifuged for 10 minutes at 1000-3000 rpm to obtain serum. Serum was then stored at ≤ -20°C for later examination using the ELISA method. The pregnancy outcome variables, birth weight and birth length were measured as soon as possible within seven days of birth using standard procedures. Body weight was measured using a digital baby scale and repeated 3 times and the average weighing result was recorded. Birth length was measured by two people using a baby length board. All data and variables were analyzed using the SPSS program. Data were analyzed using unpaired t test.

RESULTS

The total sample of the study was 56 samples consisting of 30 samples in group 1 (IFA) and 26 samples in group 2 (Moringa + IFA). Most pregnant women aged 20-30 years were 47 people (83.93%) and housewives were 38 (67.68%). Most of husbands work as entrepreneurs were 23 people (41.07%) and 47 (83,92%) had low income <2 million as shown in table 1.

Based on table 2, MDA level in the IFA group was 49.25 nmol/ml, while the Moringa+IFA group was 47.58 nmol/ml with a value of Δ = 1.67 nmol/ml with *p*-value of 0.079. Because the *p*- value > 0.05, there is no significant difference in the average MDA level in each group. The

Table 1: Characteristics of respondents.

Characteristics	Subjects (n=56)		Total N=56 (%)
	IFA n=30 (%)	Moringa n=26 (%)	
Age			
< 20 years	5 (16,67)	4 (15,58)	9 (16,07)
20-30 years	25 (83,33)	22 (84,51)	47 (83,93)
Mother`s Occupational			
Work	9 (30,0)	9 (34,62)	18 (32,14)
Doesn`t work	21 (70,0)	17 (65,38)	38 (67,68)
Father`s Occupational			
Self Employed	12 (40,0)	11 (53.6)	23 (41,07)
Farmer	10 (33,33)	4 (25.0)	14 (25,0)
Civil Servant	6 (20,0)	10 (19.6)	16 (28, 57)
Doesn`t Work	2 (6,67)	1 (1.8)	3 (5,35)
Mother`s Education			
Low	1 (3,33)	1 (3,85)	2 (3,57)
High	29 (96,67)	25 (96,15)	54 (96,43)
Father`s Education			
Low	5 (16,66)	5 (19,23)	10 (17,86)
High	25 (83,33)	21 (80,77)	46 (82,14)
Family Income			
< 2 million	24 (92,3)	23 (88,46)	47 (83.92)
> 2 million	6 (5,7)	3 (11,53)	9 (6, 08)

Source: Primer Data 2022

Table 2: The average levels of MDA, birth weight and birth length in the IFA and Moringa groups.

Variable	IFA	Moringa + IFA	Δ	p-value*
	Mean±SD	Mean±SD	Mean±SD	
MDA	49.25±0.78	47.58±8.35	1.67±7.6	0,079
Birth weight	2993.7±492.3	3042.3±411.5	48,33±80,84	0,596
Birth length	48.23±2.32	48.62±1.67	0,39±0,65	0,399

**p*-values were calculated using unpaired t-test

Table 3: The average of MDA levels during pregnancy for birth weight.

Variable	Birth Weight		Δ	p-value*
	< 2500 gram	≥ 2500 gram		
	Mean±SD	Mean±SD	Mean±SD	
MDA	49.48±0,68	48.50±5,93	0,98±5,25	0,725

**p*-values were calculated using unpaired t-test

Table 4: The average of MDA during pregnancy for birth length.

Variable	Birth Length		Δ	p-value*
	< 48 cm	≥ 48 cm		
	Mean±SD	Mean±SD	Mean±SD	
MDA	49.13±2.32	48.62±1.67	0,51±0,65	0,663

**p*-values were calculated using unpaired t-test

average of birth weight in the IFA group was 2993.7 gram while in the IFA+Moringa group was 3042.3 gram with a value of Δ = 48.33. Based on statistical tests obtained *p*-value of 0.596. There is no significant difference in the average birth weight level in each group. The average of birth length in the IFA group was 48.23 cm, while in the IFA+Moringa group was 48.62 cm with a value of Δ = 0.39. Based on the statistical test, the *p*-value was 0.399. There is no significant difference in the average of birth length levels in each group.

Based on table 3, it shows that MDA levels during pregnancy in babies with birth weight < 2500 gram was 49.48 nmol/ml higher than birth

weight ≥ 2500 gram which was 48.50 nmol/ml with a large difference of Δ= 0.98 nmol/ml. Based on statistical tests, it was obtained that *p*-value = 0.725 (> 0.05) means that there is no effect of MDA levels on birth weight.

Based on table 4, it shows that MDA levels during pregnancy in babies born with birth length <48 cm was 49.13 nmol/ml higher than birth length ≥ 48 cm which was 48.62 nmol/ml with a large difference of Δ = 0.5 nmol/ml. Based on statistical tests, it was obtained that *p*-value = 0.663 (> 0.05) means that statistically there is no effect of MDA levels on birth length.

DISCUSSION

The results of this study indicate that statistically, there is no significant difference in MDA levels in the two groups. However, the MDA level in the group that was only given IFA was higher than that given the combination of IFA and Moringa. In several studies it has been reported that moringa contains strong non-enzymatic antioxidant components to scavenge free radicals and prevent oxidative DNA damage. Moringa leaf extract also contains several micronutrients including vitamins C, E and A, iron, zinc, folic acid and selenium which work as enzyme cofactors that play an important role in DNA synthesis and repair and prevent DNA oxidative damage.[17] Moringa leaf extract contains polyphenols, flavonoids and phenols as antioxidant components that protect cells from free radicals. Metabolism of polyphenols can neutralize free radicals by donating electrons or hydrogen atoms to inhibit free radical generation or by inactivating free radical precursors. Polyphenols such as Fe2+ and directly reduce the Fenton reaction thereby preventing oxidation by hydroxyl radicals.[18]

This study is in line with research conducted on non-anemic pregnant women comparing moringa leaf extract and IFA to MDA levels in pregnant women. The results of this study showed that there were significant differences in MDA levels in the two groups where MDA levels in the control group (IFA) were higher than the intervention group (Moringa leaf extract).[19] Malondialdehyde (MDA) is a biomarker for lipid peroxidation that is also used to evaluate biochemical end points in response to oxidative stress. Based on the results of this study, it was found that there was a significant decrease in MDA levels and 8-OHdG levels in the honey + moringa treatment group so that there was a significant effect on giving honey and Moringa leaf extract in preventing DNA damage in passive smoking pregnant women.[20]

Moringa oleifera leaf powder (MLOP) supplementation increased plasma total antioxidant capacity (TAC) by decreasing malondialdehyde (MDA) and total oxidant status (TOS) in contrast to the control group. Superoxide dismutase (SOD) and peroxidase (POD) activities were increased in the two supplemented groups, whereas catalase (CAT) activity was very high in the MOLP supplemented group.[21] There was a significant decrease in MDA levels in the group that received moringa leaf extract and royal jelly.[16] Moringa capsules and pumpkin seed biscuits also contribute to preventing increased MDA levels thereby preventing stress, low birth weight, preeclampsia and reducing maternal and infant mortality.[22] This is in contrast to research which showed that there was no significant difference in 8-OHdG levels between the group receiving moringa leaf extract and IFA and the group receiving only IFA.[23]

In this study, the average of birth weight and birth length results were higher in the group that was given the moringa leaf extract intervention compared to the group that was only given IFA. The results of this study are in line with research[24] which states that giving 2g of moringa leaf powder supplementation every day for two months during the third trimester of pregnancy is proven to increase child birth weight in moderately anemic pregnant women and improve markers of pregnant women's health status. Administration of Moringa leaf powder tends to protect against stunting but not against underweight.[13] When given to pregnant women, MO extract reduced the chances of their child being stunted.[25] the MO intervention group had a lower incidence of LBW than blood supplement pills, as well as results for birth length and head circumference, which could prevent stunting and increase the amount and quantity of breast milk but not the quality of breast milk and LILA.[26-28]

MDA levels during pregnancy were higher in women who have babies with birth weight < 2500 grams and birth length < 48 cm. Maternal or fetal oxidative stress plays an important role in the pathophysiology of low birth weight. The increased metabolic rate to ensure adequate fetal growth simultaneously increases the levels of oxidative stress in the placental tissue. Simultaneously, an increase in mitochondrial activity causes an increase in ROS levels, especially in the syncytiotrophoblast.[29] Oxidative stress is a significant contributor to the etiology of unfavorable pregnancy outcomes, defined as an imbalance between free radical production and antioxidant defenses. Oxidative stress affects the antioxidant capacity of the placenta and reduces the system by increasing antioxidant consumption and decreasing antioxidant levels. Oxidative stress causes damage to DNA, proteins, and lipids in the placental tissue, which accelerates the aging process. Fetal survival is affected by premature aging of the placenta, which is associated with placental insufficiency, which prevents the organs from meeting the needs of the fetus.[3] A study to examine the clinical utility of serum homocysteine and MDA as markers of oxidative stress in pregnant women who had complications of abortion and preterm birth suggested that homocysteine levels and MDA concentrations were significantly increased in pregnant women who had abortions and gave birth to premature babies compared to the control group with a p-value =0.040 and p=0.031.[30]

The limitations of this study was the measurement of MDA levels was only done once so we could not see the difference between MDA levels before and after the intervention of Moringa leaf extract. The decrease in MDA levels which was not significant in the treatment group (Moringa + IFA) was possibly due to the administration of Moringa leaf extract along with IFA. High iron supplementation, especially in non-aemic pregnant women, will trigger increased oxidative stress.[31] Ferrous iron used as a supplement in pregnant women is a pro-oxidant which increases the production of free radicals thereby increasing lipid peroxidation in anemic and non-anemic pregnant women.[32]

In conclusion, oxidative stress is related to poor pregnancy outcomes. Giving Moringa leaf extract has the potential to prevent oxidative stress in pregnant women and improve pregnancy outcomes so that Moringa leaf extract can be used as an additional supplement for women from the preconception period. Further research needs to be carried out in a larger number of samples using Moringa leaf extract without the administration of IFA.

ABBREVIATIONS

ANC: Antenatal care

CAT: Catalase

DNA: Deoxyribo nucleic acid

ELISA: Enzyme-link immune sorbent

GABA: Gamma-aminobutyric acid

IFA: Iron-folic acid

IUGR: Intra uterine growth restriction

LBW: Low birth weight

MDA: Malondialdehyde

MOLP: Moringa oleifera leaf powder

MO: Moringa oleifera

PrCarb: Protein carbonyl

RNS: Reactive nitrogen spesies

ROS: Reactive oxygen spesies

SOD: Superoxide dismutase

TAC: Total antioxidant capacity

TOS: Total oxidant status

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AUTHOR`S CONTRIBUTIONS

RR contributed in data collection and manuscript drafting. VH, AA and AS provided general input throughout the design, analysis and writing processes of this manuscript. AF performed statistical analysis. HH and MR contributed in data collection. AM was the final internal reviewer who contributed in reviewing and editing manuscript. All authors read and approved the final manuscript.

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AVAILABILITY OF DATA AND MATERIALS

The primary data for this study is available from the authors on direct request. Please contact author for data requests.

DECLARATIONS

Ethics approval and consent to participate: This study was approved by Ethics Committee of Public Health Faculty, Hasanuddin University with the recommendation number of ethics approval: 4885/UN4.14.1/TP.02.02/2021.

Consent for publication: All patients provided written informed consent to participate in the study.

Competing interest: The authors declare that they have no competing interests.

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