RESEARCH ARTICLE

Role of natural antioxidant after exercise in reducing malondialdehyde (MDA) levels during pregnancy

Ocktariyana, Sari Wahyuni

Abstract

Objective: This study aimed to analyse the effect of a supplementary treatment with natural antioxidants after exercise on the MDA levels in women during the third trimester of pregnancy.

Methods: This experimental study from August to October 2016 at several midwife practices and community health centres (puskesmas) in Palembang, Indonesia included 24 prenatal pregnant women separated to 3 groups (n = 8); a control group, a positive control group that undertook an exercise programme, and a treatment group that underwent exercise programme and took natural antioxidants after the exercise. Guava juice containing vitamin C was used as antioxidant treatment. The MDA levels were analysed by spectrophotometry.

Results: The MDA levels were significantly different among the groups ($p < 0.001$). The LSD test confirmed a difference between serum MDA levels in the control, exercise and exercise + antioxidant groups (2.89 ± 0.53, 2.11 ± 0.31 and 1.47 ± 0.23 nmol/mL, respectively).

Conclusion: The MDA levels were significantly decreased after combined treatment with natural antioxidants and exercise in the third trimester of pregnancy.

Keywords: Antioxidant, Malondialdehyde, MDA level, Third trimester of pregnancy, Exercise.

(JPMA 71: S-14 [Suppl. 2]; 2021)

Introduction

Pregnancy is a condition that is vulnerable to many types of stress that can affect physiology and metabolic function. Pregnancy is also associated with an increase in the energy and oxygen needs of the mother.[1,2] Metabolic processes that increase the use of oxygen; furthermore, if the available oxygen is not used at most, this may cause oxidative stress and contribute to an excessive production of free radicals, with negative consequences for the continuation of the pregnancy process.[3] An imbalance between antioxidants and free radicals in pregnancy can cause pathological changes that can lead to pregnancy complications. For this reason, the availability of a biochemical marker of antioxidant and oxidant status is beneficial for indentification of complications that may occur during pregnancy.[4]

Exercise is one therapy that is intended to alleviate problems that occur during pregnancy due to the formation of free radicals, but exercise programmes must be adapted to meet the abilities of pregnant women.[5,6] Physical exercise during pregnancy can control weight gain, prevent diabetes and hypertension and minimise work time.[7] Exercising by engaging in sports activities is one way to neutralise the formation of free radicals because excess oxygen can be consumed to produce the energy required for sports physical activities.[8]

Exercise may also reduce the level of malondialdehyde (MDA), a biochemical marker associated with free radical stress. Malondialdehyde (MDA) is formed as a result of lipid peroxidation due to free radicals, and its presence can, therefore, be used as an oxidative stress indicator.[1] Previous research has reported higher MDA levels in pregnant than in non-pregnant women, and rises in MDA levels correspond to increases in gestational age during the first, second, and third trimesters.[9,10] Other researchers have also found significant increases in MDA levels in pregnant women with hypertension, as well as an accompanying decrease in non-enzyme antioxidants, such as vitamins E, C and A.[11-14]

This background knowledge has led to the current work, which focuses on the potential benefits of giving antioxidants to lower MDA levels in the third trimester of pregnancy. This aim of the study was to analyse the effect of natural antioxidants on MDA levels in women in the third trimester of pregnancy following exercise.

Subjects and Methods

This experimental study with a post-test-only control group was carried out from August to October 2016 at several midwife practices and community health centres

Department of Midwifery, Politeknik Kesehatan Palembang, Ministry of Health, Indonesia.

Correspondence: Sari Wahyuni. Email: sariwahyuniplg@gmail.com

(puskesmas) in Palembang and at the Bio Sains Riset Laboratory, Palembang.

The samples of this study had informed consent as well as ethical clearance approval number 007/UP-ETIK/I/2016, and clinical trial ID number is NCT04226183. The total samples were collected from 24 prenatal pregnant women and divided into three groups of 8 women each: a control non-exercise group, a supportive control group that participated in the exercise programme and a treatment group that participated in the exercise programme and drank guava juice as natural antioxidants after exercise.

The samples were prepared before they were sent to the laboratory to determine the MDA level. Each blood samples (4 cc) were taken from the median cubital vein using a 5 cc syringe and immediately transferred to a micro centrifuge tube containing anticoagulants. The whole blood was centrifuged at 3500 rpm for 5 minutes to separate the plasma serum. The plasma was transferred to a new micro centrifuge and stored at -70°C before being sent to the laboratory.

Antioxidant compounds have been administered in the form of guava juice. The subject as treatment group consumed 250 ml guava juice without additional sugar.

The materials used in the study were 5 cc syringes, cotton, alcohol, syringes, disposable gloves, blood tubes that contain anticoagulant, serum, 200ul of pipette size, tips, stir bar, polyphlinrifuge tube, Spectrophotometer, vortex, magnetic stirrer, water bath, 5% of 2-thiobarbiturat acid (TBA), glacial acetic acid, sodium hydroxide, Malondialdehyde standard solution, 0.65% of Natrium thiobarbiturate, and distilled water.

The MDA levels were determined by a spectrophotometric assay at the Bio Sains Riset Laboratory in Palembang. Preparation of the reagents began by dissolving 0.67 g of 2-thiobarbiturate acid in 100 ml distilled water, then by adding 0.5 g of sodium hydroxide and 100 ml glacial acetic acid, and by adding 125 ul of stock solution dissolved in aquabidest.

For MDA analysis, 100 UL samples (blood plasma) or standards were inserted into the labelled tubes and 0, 9ml distilled water was added om each of the tubes. On the next sample TBA reagent 0.5ml was added. The tube contains a solution then heated in a water bath at a temperature of 95^{o}C for 1 hour and further centrifuged at 7000 rpm for 10 minutes. The absorbance was read at 550 nm with a spectrophotometer.

The group results were compared by one-way ANOVA, with $p < 0.05$ indicating statistical significance using SPPS version 16.0.

Results

In this analysis, initial parametric tests were carried out to validate the normal distribution of the data using the Shapiro-Wilk test (Table-1). All data had a normal distribution.

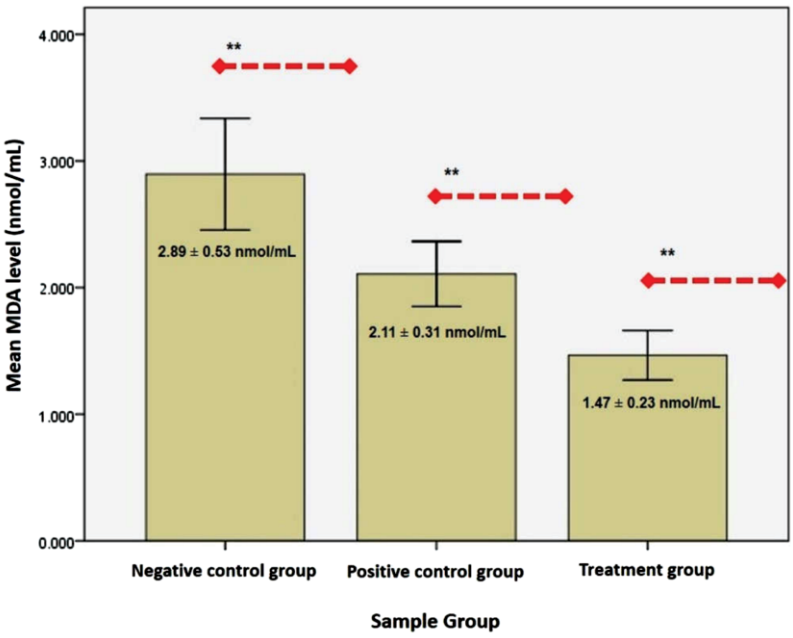

Figure: Effects of exercise and exercise plus antioxidant treatment on the mean serum MDA levels in women in the third trimester of pregnancy ($p < 0.01$).

Table-1: Test results for the normality of MDA levels.

Observation Group	P-value	Distribution	Observation Group	P-value
Control group (no exercise or antioxidants)	0.291	Normal	Negative control	0.291
Exercise group	0.585	Normal	Positive control	0.585
Exercise + antioxidant group	0.233	Normal	Treatment Group	0.233

A p-value $\geq$ 0.05 indicates a normal distribution of the data.

Table-2 Effects of exercise and antioxidants on MDA levels in pregnant women in the third trimester.

Treatment	N	Serum MDA level (nmol/mL)	P value
Control group (no exercise or antioxidants)	8	2.89 ± 0.53 a	< 0.001
Exercise group	8	2.11 ± 0.31 b	
Exercise + antioxidant group	8	1.47 ± 0.23 c	

Values with different letters indicate significant differences (p-value < 0.05).

Table-1 shows the Shapiro-Wilk test results for the MDA levels for each group. The p-value values were all greater than the significance level $\alpha = 0.05$, so that all data met the parametric prerequisite test for normal distribution and could be further evaluated by parametric statistical tests to prove the research hypothesis.

The MDA levels differed significantly in the three groups (p value < 0.001). The LSD test confirmed a statistically significant difference for the MDA levels of the control group (2.89 ± 0.53 nmol/mL), exercise group (2.11 ± 0.31 nmol/mL) and exercise + antioxidant group (1.47 ± 0.23 nmol/mL).

Table-2 confirms that the third trimester MDA levels were lower in pregnant women who were engaged in pregnancy exercises and that there were further significant reductions in MDA levels when exercise was combined with an antioxidant treatment. These data are shown in Figure-1. The lowest mean MDA level (1.47 ± 0.23 nmol/mL) was observed in the treatment group which combined both exercise and antioxidant intake.

Discussion

In this study, the effect of treatment with natural antioxidants on serum MDA levels in women in the third trimester of pregnancy was analysed. The working hypothesis was that oxidative stress arises when an imbalance exists between the production of oxidants and antioxidants in the body and that MDA, which is an end product of stress-related lipid peroxidation, [15] would be a useful biological marker for oxidative stress in pregnant women.

Free radicals are more harmful to cells than other non-free radical oxidants since free radicals have a highly reactive nature and appear to undergo chain reactions that promote the formation of additional new radicals. Antioxidants can prevent damage from free radicals by squeezing this reactivity and supplying electrons to reduce the adverse effects of free radicals such as superoxide radical (SOR).[16] In the previous study, pregnant women who received high oxygen levels (60%) during Caesarean section with regional anaesthesia (subarachnoid block) were found to have high levels of free radicals in both maternal blood and the blood of the newborn when compared with mothers given normal levels (21%) of oxygen.[17] Blood gas and MDA levels were reviewed every 10 minutes, and a significant increase in MDA levels was observed in the 20th minute.

In the present study, the MDA level was decreased in the third trimester of pregnancy which was consumed natural antioxidant as well as exercise. The antioxidant compounds used in this study were natural ingredients found in guava fruit juice, containing substantial amounts of vitamin C (ascorbic acid). Natural antioxidant compounds are electron-donating compounds and may include enzymes and protein-binding metals.[18] Antioxidants function in a 2-stage strategy: they prevent the accumulation of oxidant compounds in excess, and they disrupt the occurrence of chain reactions that produce free radicals. Oxidative stress occurs when the resulting SOR level is higher than the level that can be suppressed by cell defence mechanisms. If free radical compounds are not extinguished, oxygen will a poison in the body. Antioxidants donate electrons to reduce the negative impacts of SOR.[19]

Vitamin C is an essential micronutrient that is needed for normal metabolic reactions in the body. Vitamin C must be obtained from the diet in humans, with the primary source being fresh fruit, particularly citrus fruits and vegetables. Some studies have reported lower levels of non-enzyme antioxidants, namely vitamins C and E, in amniotic fluid, foetal cord blood and maternal blood in long-term labour that ended with Caesarean section than in normal labour.[20-22]

The study also reported that the growing increase in MDA levels is accompanied by a decrease in non-enzyme antioxidants, such as vitamins E, C and A, in pregnant women with hypertension.[11-13] Pregnancy complications in the first, second and third trimesters can arise due to the formation of reactive oxygen species and the psychological burden of pregnancy. Nevertheless, regardless of their cause, these risks can be reduced or eliminated by offering care and sporting activities during pregnancy. The treatments are expected to eliminate physical complaints, while exercise during pregnancy may prevent the formation of free radicals. However, exercise programmes must always take into account the skill of pregnant women.[5,6]

In this study, MDA levels were higher in third-trimester pregnant women that did not participate in a pregnancy exercise programme than in those who did. This finding

was consistent with the results of a study of pregnant women engaged in pregnancy gymnastics exercises beginning after 20 weeks of gestation. In the group that participated in the exercise programme, MDA levels were substantially lower than those of the control group that did not participate ($p < 0.05$).[11] Another study also showed that the labour process could be greatly changed the use of pregnancy exercises, as mothers who participated in pregnancy exercises went through the delivery process more efficiently and quickly than mothers who did not undertake pregnancy exercises.[6]

In addition, the development of free radicals in the body and the formation of reactive oxygen species are physiological processes. When free radical levels increase, the body tries to overcome this situation by producing several antioxidants, known as counteracting antioxidants.[1,2] Sporting activities are one way to neutralise the formation of free radicals as physical activity guides the use of excess oxygen to the production of metabolic energy rather than towards the generation of free radicals.[8] The duration and frequency of the exercise programme can therefore inversely affect the formation of free radicals, and these effects can be controlled by measuring changes in the levels of biochemical markers such as MDA.

Conclusion

A combination of exercise and intake of natural antioxidants significantly reduced MDA levels in women during the third trimester of pregnancy.

Acknowledgement: The authors thank to Department of Midwifery, Politeknik Kesehatan Palembang, Ministry of Health, Indonesia for the award of a Beginner Lecturer Research Grant in 2016.

Disclaimer: None

Conflict of Interest: None

Funding Disclosure: This study was supported by Department of Midwifery, Politeknik Kesehatan Palembang, Ministry of Health, Indonesia.

References

1. Patil SB, Kodliwadmath MV, Kodliwadmath SM. Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. J Obstet Gynecol India 2006;56:399-401.
2. Patil SB, Kodliwadmath MV, Kodliwadmath SM. Study of oxidative stress and enzymatic antioxidants in normal pregnancy. Indian J Clin Biochem 2007;22:135-7. doi: 10.1007/BF02912897.
3. Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. J Nutr 2003;133(Suppl 2):s1700-8. doi: 10.1093/jn/133.5.1700S
4. Jenkins C, Wilson R, Roberts J, Miller H, McKillop JH, Walker JJ. Antioxidants: their role in pregnancy and miscarriage. Antioxid Redox Signal 2000;2:623-8. doi: 10.1089/15230860050192369.
5. Kaiser LL, Allen L. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. J Am Diet Assoc 2002;102:1479-90. doi: 10.1016/s0002-8223(02)90327-5.
6. Walker JJ. Pre-eclampsia. Lancet 2000;356:1260-5. doi: 10.1016/S0140-6736(00)02800-2.
7. Impact of physical activity during pregnancy and postpartum on chronic disease risk. Med Sci Sports Exerc 2006;38:989-1006. doi: 10.1249/01.mss.0000218147.51025.8a.
8. Plowman SA, Smith DL. Exercise Physiology: for Health, Fitness, and Performance, 2nd ed. Philadelphia, USA: Lippincott Williams & Wilkins, a Wolters Kluwer Business, 2003; pp 636.
9. Arribas L, Almansa I, Miranda M, Muriach M, Romero FJ, Villar VM. Serum Malondialdehyde Concentration and Glutathione Peroxidase Activity in a Longitudinal Study of Gestational Diabetes. PLoS One 2016;11:e0155353. doi: 10.1371/journal.pone.0155353.
10. Toescu V, Nuttall SL, Martin U, Kendall MJ, Dunne F. Oxidative stress and normal pregnancy. Clin Endocrinol (Oxf) 2002;57:609-13. doi: 10.1046/j.1365-2265.2002.01638.x.
11. Kobe H, Nakai A, Koshino T, Araki T. Effect of regular maternal exercise on lipid peroxidation levels and antioxidant enzymatic activities before and after delivery. J Nippon Med Sch 2002;69:542-8. doi: 10.1272/jnms.69.542.
12. Ilhan N, Ilhan N, Simsek M. The changes of trace elements, malondialdehyde levels and superoxide dismutase activities in pregnancy with or without preeclampsia. Clin Biochem 2002;35:393-7. doi: 10.1016/s0009-9120(02)00336-3.
13. Yoneyama Y, Sawa R, Suzuki S, Doi D, Yoneyama K, Otsubo Y, Araki T. Relationship between plasma malondialdehyde levels and adenosine deaminase activities in preeclampsia. Clin Chim Acta 2002;322:169-73. doi: 10.1016/s0009-8981(02)00175-4.
14. Uboh FE, Ebong PE, Oton E, Itam IH, Barnaby N. Antioxidant vitamins and free radical status in Nigerian preeclamptic women. Res J Obstet Gynecol 2008;1:30-3.
15. Gaetani GF, Kirkman HN, Mangerini R, Ferraris AM. Importance of catalase in the disposal of hydrogen peroxide within human erythrocytes. Blood 1994;84:325-30.
16. Khaw KS, Wang CC, Ngan Kee WD, Pang CP, Rogers MS. Effects of high inspired oxygen fraction during elective caesarean section under spinal anaesthesia on maternal and fetal oxygenation and lipid peroxidation. Br J Anaesth 2002;88:18-23. doi: 10.1093/bja/88.1.18.
17. Suryohudoyo P. Oxidants, Antioxidants and Free Radicals. [Online] 2000 [Cited 2020 October 18]. Available from URL: https://docplayer.info/30636838-Oksidan-antioksidan-dan-radikal-bebas.html
18. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002;82:47-95. doi: 10.1152/physrev.00018.2001
19. Beinder E, Scalera F, Schlembach D. Influence of reduced intracellular glutathione availability on the secretion of vasoactive substances by human umbilical vein endothelial cells. Hypertens Pregnancy 2001;20:45-56. doi: 10.1081/PRG-100104171.
20. Chaudière J, Ferrari-Iliou R. Intracellular antioxidants: from chemical to biochemical mechanisms. Food Chem Toxicol 1999;37:949-62. doi: 10.1016/s0278-6915(99)00090-3.
21. Woods JR Jr, Cavanaugh JL, Norkus EP, Plessinger MA, Miller RK. The effect of labor on maternal and fetal vitamins C and E. Am J Obstet Gynecol 2002; 187:1179-83. doi: 10.1067/mob.2002.127131.