

THE EFFECT OF GLUTATHIONE SUPPLEMENTATION (L-GLUTATHIONE, VITAMIN C, ALPHA LIPOIC ACID, AND ZINC) ON TOTAL ANTIOXIDANT STATUS (TAS) LEVEL

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ABSTRACT Introduction: Reactive oxygen species (ROS) are produced continuously in our body as a result of biochemical reactions which may cause oxidative stress and various dysfunctions. Antioxidants are substances which are postulated to prevent or even overcome the adverse effects of free radicals. Glutathione is a type of antioxidant that is required for maintaining mitochondrial function, DNA health, improving immune function and providing anti-inflammatory effects. However, the benefit of glutathione as a health supplement in healthy individuals is yet to be elucidated. **Method:** A total of 30 healthy women was included in this double-blinded randomized controlled trial study. Subjects were randomly allocated into two groups receiving placebo and antioxidant capsule, respectively. One capsule of antioxidant contains 500 mg L-glutathione, 250 mg ascorbic acid, 50 mg alpha-lipoic acid (ALA), and 4 mg zinc which was given every day to the treatment group for 12 weeks. The total antioxidant serum (TAS) level before and after 12 weeks of treatment was examined using ELISA. **Result:** No significant TAS level difference was observed between both groups before and after treatment ($p > 0.05$). Also, there was also no significant difference in TAS level within the group before and after 12 weeks of treatment ($p > 0.05$). No significant side effects were observed. **Conclusion:** This study shows that short-term administration of antioxidant supplement containing glutathione, ascorbic acid, ALA, and zinc did not significantly affect the antioxidant level.

KEYWORDS alpha lipoic acid, glutathione, total antioxidant status, vitamin C, zinc

Introduction

Reactive oxygen species (ROS) are produced continuously in our body as a result of biochemical reactions. If the production of ROS and other free radicals exceeds the capacity of endogenous

antioxidants, a condition called oxidative stress would occur. Excessive ROS will damage cellular lipids, proteins and DNA and inhibit normal cell function, which can cause various diseases, such as cancer, hypertension, diabetes, atherosclerosis, and premature ageing [1],[2]

Antioxidants are substances which can inhibit or delay the oxidation of various substrates. Endogenous antioxidant substances consist of non-enzymatic, such as uric acid, glutathione, bilirubin, thiols, albumin, and nutritional factors, including vitamins and phenols) and enzymatic, such as superoxide dismutase (SOD), glutathione peroxidases [GSH-PX], catalase, and glutathione reductase (GSHR). When antioxidant defences weaken, body cells and tissues become more susceptible to deterioration and disease. Therefore, maintaining antioxidant levels at an

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adequate level is essential in preventing or even overcoming various types of diseases.[3],[4]

Glutathione is a low molecular weight thiol which has an essential role in maintaining the intracellular redox balance. Glutathione is essential for maintaining mitochondrial function, DNA health, and energy production. Also, it is also beneficial in improving immune function and providing anti-inflammatory effects.[5] This is augmented by the fact that healthy subjects are shown to have optimal glutathione levels.[6]

However, the benefit of glutathione given as a health supplement in healthy individuals is yet to be elucidated. Most studies were done on subjects with diseases[7],[8] and to our knowledge, only one study was done in healthy subjects[9]. This study aims to examine the effect of antioxidant supplementation on the total antioxidant status (TAS) level in healthy subjects.

Methods

Study Design

The double-blinded randomized controlled trial study was conducted in the Dermatovenereology Department, Faculty of Medicine, Hasanuddin University from May until July 2018 and has obtained approval from the Institutional Ethical Review Board.

Subjects

A total of 30 healthy volunteers aged 30-65 years old were included in this study. All patients were healthy subjects. Informed consent was obtained from each subject. Patients with history or family history with skin cancer, chronic systemic disease, supplementation containing glutathione or other antioxidants, and smoking were excluded. The sampling method used was consecutive sampling.

Study Protocol

Subjects were randomly allocated into two groups using block randomization method. One capsule of antioxidant containing 500 mg L-glutathione, 250 mg ascorbic acid, 50 mg alpha-lipoic acid (ALA), and 4 mg zinc was given every day to the treatment group for 12 weeks. The other group was given a placebo capsule with similar size, shape, and colour for the same duration. Venipuncture was done in each subject to measure the total antioxidant serum (TAS) level before and after 12 weeks of treatment. The TAS level examination was carried out using ELISA with human total antioxidant capacity (T-AOC) kit (Bioassay Technology Laboratory) following the instruction from the manufacturer.

Statistical Analysis

The data in this study were analyzed using the Statistical Package for Social Sciences (SPSS) 21.0 for Windows (SPSS Inc. Chicago, IL, USA). Results were presented in the form of tables and charts. Wilcoxon test was used to compare the median of TAS level in each before and after treatment. The difference of TAS level between both groups was assessed using the Mann-Whitney test. A p-value of less than 0.05 was considered significant.

Results

A total of 30 subjects were included in this study. Most subjects (86.67%) were between 30-45 years old and worked as a cleaning service (Table 1).

Table 1 Characteristics of Study Population

Variable		(n)	%
Age*	30- 45 years	26	86.67
	46- 65 years	4	13.33
Occupation	Cleaning Service	20	66.67
	Office workers	7	23.33
	Housewife	3	10

Table 2 TAS Level in Each Group Before and After Treatment

Median TAS Level (Min-Max)				
Group	Before Treatment	After Treatment	Change in TAS	p-value
Treatment	15,78 (4,7-41,6)	13.58 (4,7-41,6)	2,201	0,156*
Control	16,21 (2,2-84,6)	17.01 (2,2-84,6)	-0,802	0.955*
p-value	0.744**	0.285**		
*Wilcoxon test				
**Mann-Whitney test				

Table 2 shows the TAS level in each group before and after treatment. No significant TAS level difference was observed between both groups before and after treatment ($p > 0.05$). Also, there was also no significant difference in TAS level observed within the group before and after 12 weeks of treatment ($p > 0.05$).

No serious adverse effects were found in this study. Two subjects and three subjects reported a mild headache and epigastric pain, respectively.

Discussion

The study involved 30 women aged 30 - 65 years who were divided into two groups, namely group A (treatment) and B (placebo control). Table 1 shows the population demographic data of this study. Pre-menopausal women were taken to avoid bias due to the effects of oxidative stress that occurred at menopause. Low estrogen levels in menopause have a pro-oxidant effect, where the breakdown of genetic material, the formation of DNA adducts, and alkaline oxidation occur and increases the level of free radicals.[10] Also, increased oxidative stress in menopause is also shown through increased levels of pro-inflammatory cytokines.[11]

Table 4 shows that the TAS levels decreased by 2,201 from 15,785 to 13,584 (4.5-41,0) in the treatment group and increased 0.802 from 16.206 to 17.008. However, these changes were not significant. Thus, the results of this study showed that administration of the antioxidant capsules for 12 weeks did not show an increase in TAS levels. A study conducted in 39 healthy adults with twice daily 500 mg oral glutathione (GSH) supplementation for 4 weeks showed a similar finding, where no significant

changes in erythrocyte glutathione concentration was observed after 4 weeks of treatment.[9] Thus, the results of our study confirm that the administration of short-term supplementation of glutathione does not affect antioxidant levels in healthy subjects.

Vidovic et al. (2014) evaluated TAS levels in 38 healthy subjects who received daily supplements of 500 mg alpha-lipoic acid for three months, where TAS levels significantly increased after treatment ($p < 0.001$). [7] The difference with the results obtained in our study can be caused by different dosage, where we used a dose of 50 mg/day. This lower dose might not be adequate to overcome the oxidative stress experienced by our population.

This study shows that 4 mg daily zinc administration for 12 weeks did not provide a significant SAT increase. A different result was obtained by Mariani et al., where a significant increase in antioxidant enzyme activity (400 U / g Hb) was observed in 1108 healthy subjects after consuming 10 mg zinc daily for seven weeks. However, the significant increase found in this study was only shown in populations with plasma zinc concentrations above 11 μ M, whereas in those with zinc level of $< 11 \mu$ M, no significant changes were found, even tending to decline.[12] In our study, the plasma zinc concentrations before supplementation were not measured and hence we could not confirm the finding of their study.

The administration of antioxidant supplements and vitamins has been believed to improve endurance and improve health (McHugh S, Moon H, 2008) However, this is yet to be supported by adequate scientific evidence. Most studies were conducted in subjects with diseases [13] while those on healthy subjects is very limited. [9] Data from our study show that short-term antioxidant supplementation does not affect antioxidant activity in healthy populations. These results are in line with a study that looked at the effects of antioxidant supplementation for 77 months in healthy populations, where no significant effect on health-related quality of life was evident. [14]

As the main component in the intervention in this study is glutathione, future studies may consider to measure the levels of intracellular glutathione as a reflection of oxidative stress. The most widely used and sensitive method is the enzyme recycling assay method, which was first performed by Tietze and modified by Adams. There are two forms of glutathione, namely the reduced sulfhydryl form (GSH) and the oxidized form, glutathione disulfide (GSSG). GSH is oxidized to GSSG and 5-thio-2-nitrobenzoic acid (TNB). GSSG is then reduced to GSH by the glutathione reductase enzyme. TNB levels are proportional to the amount of GSSG and GSH which indicates the total glutathione level. Thus, measuring TNB levels at a wavelength of 412 nm can illustrate the total glutathione. [15]

Conclusion

This study shows that short-term administration of antioxidant supplement containing glutathione, ascorbic acid, ALA, and zinc did not significantly affect the antioxidant level.

References

1. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol Pathol.* 2002;30(6):620-50.
2. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multi-gene family: a comparison of the CuZn-SOD (SOD1), Mn-

SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med.* 2002;33(3):337-49.

3. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *International journal of biomedical science: IJBS.* 2008;4(2):89.
4. Fusco D, Colloca G, Monaco MRL, Cesari M. Effects of antioxidant supplementation on the aging process. *Clin Interv Aging.* 2007;2(3):377.
5. Arjinpauthana N, Asawanonda P. Glutathione as an oral whitening agent: A randomized, double-blind, placebo-controlled study. *Journal of Dermatological Treatment.* 2012;23(2):97-102.
6. Droge W, Breitkreutz R. Glutathione and immune function. *Proc Nutr Soc.* 2000;59(4):595-600.
7. Vidovic B, Milovanovic S, Dordevic B, Kotur-Stevuljjevic J, Stefanovic A, Ivanisevic J, et al. Effect of alpha-lipoic acid supplementation on oxidative stress markers and antioxidant defense in patients with schizophrenia. *Psychiatria Danubina.* 2014;26(3):205-13.
8. Bazzini C, Rossetti V, Civello DA, Sassone F, Vezzoli V, Persani L, et al. Short-and long-term effects of cigarette smoke exposure on glutathione homeostasis in human bronchial epithelial cells. *Cell Physiol Biochem.* 2013;32(7):129-45.
9. Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. *The Journal of Alternative and Complementary Medicine.* 2011;17(9):827-33.
10. Doshi SB, Agarwal A. The role of oxidative stress in menopause. *Journal of mid-life health.* 2013;4(3):140.
11. Santo Signorelli S, Neri S, Sciacchitano S, Di Pino L, Costa MP, Marchese G, et al. Behaviour of some indicators of oxidative stress in postmenopausal and fertile women. *Maturitas.* 2006;53(1):77-82.
12. Mariani E, Mangialasche F, Feliziani F, Cecchetti R, Malavolta M, Bastiani P, et al. Effects of zinc supplementation on antioxidant enzyme activities in healthy old subjects. *Exp Gerontol.* 2008;43(5):445-51.
13. Mazani M, Argani H, Rashtchizadeh N, Ghorbanhaghjo A, Hamdi A, Estiar MA, et al. Effects of zinc supplementation on antioxidant status and lipid peroxidation in hemodialysis patients. *J Ren Nutr.* 2013;23(3):180-4.
14. Briançon S, Boini S, Bertrais S, Guillemin F, Galan P, Hercberg S. Long-term antioxidant supplementation has no effect on health-related quality of life: The randomized, double-blind, placebo-controlled, primary prevention SU. VI. MAX trial. *Int J Epidemiol.* 2011;40(6):1605-16.