Original Article

The levels of lycopene, α-tocopherol and a marker of oxidative stress in healthy northeast Thai elderly

Prasit Suwannalert MSc[1], Patcharee Boonsiri PhD[1], Tueanjit Khampitak MD[1], Kovit Khampitak MD[2], Pote Sriboonlue MSc[1] and Puangrat Yongvanit DrScAgr[1]

[1]*Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[2]*Department of Obstetrics and Gynecology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

An imbalance between oxidative stress and antioxidant capacity has been proposed to play an important role in the development and progression of chronic diseases in the elderly. The present study was carried out to investigate correlation between the serum antioxidants (lycopene and α-tocopherol) and malondialdehyde (MDA), a marker of oxidative stress in the healthy Thai elderly. The 207 healthy subjects aged 60-91 years old (72 males and 135 females) in Khon Kaen province, Thailand were enrolled in this study. They were interviewed by questionnaires about smoking habit. Serum lycopene and α-tocopherol levels were determined by high performance liquid chromatography (HPLC). MDA was measured by thiobarbituric assay. Serum lycopene and α-tocopherol levels in the elderly were 0.27 μmol/L (95% CI = 0.23-0.31) and 22.10 μmol/L (95% CI = 20.99-23.22), respectively. Males had significant lower serum lycopene and α-tocopherol levels than females (p<0.01). Of 72 males, 31.94% are current smokers whereas 1.4% of 135 females are current smokers. Current smokers had significantly lower serum lycopene (0.17 ± 0.11 μmol/L) than current non-smokers (0.28 ± 0.27 μmol/L) (p=0.04) but level of α-tocopherol had non significance (p=0.21). Moreover, the current smokers had higher malondialdehyde level (1.55 ± 0.10 μmol/L) than the current non-smokers (1.35 ± 0.04 μmol/L) (p=0.09). Thus, dietary antioxidant supplementation from local fruits and vegetables may have a beneficial role in the prevention of chronic diseases at high-risk oxidative stress such as smoking in these elderly.

Key Words: lycopene, α-tocopherol, oxidative stress, smoking, northeast Thais

Introduction

The elderly are increasing in numbers and have a long life expectancy in most parts of the world. It is important to be aware of health of the elderly. A thorough nutrition status may be basic to understand the elderly total health. The oxidant-antioxidant system is balance in healthy conditions.[1] Prooxidants and antioxidants maintain a ratio and shift in this ratio towards prooxidants gives rise to oxidative stress. Excess of free radicals damage in the body has been proposed to play a crucial role in abnormal gene expression, immunity perturbation, and mutagenesis.[2] This oxidative stress may be either mild or severe depending on the extent of shift and remains the cause of several diseases such as cardiovascular diseases, neurological diseases, malignancies, renal diseases, and diabetes.[1,2] Taking a study of their micronutrient antioxidant status before undertaking any medical procedure is mandatory in order to provide appropriate care for the elderly because several recent studies have shown a correlation between antioxidant status and diseases. Lycopene and α-tocopherol have been shown to be associated with a lower risk of chronic diseases such as cardiovascular diseases and cancers.[3] The level of these serum antioxidants may be turned down in the elderly because they may be used to quench the free radicals, especially in people who have risk factor of oxidative stress such as smoking.[4,5] Cigarette smoke is a potent exogenous source of oxidative stress, one potential mechanism for its untoward health effects.[6] The tobacco smoke intake by the smoker is a toxic product and a risk factor for diseases as cancer (lung, pharyngeal, laryngeal and esophageal, kidney, and bladder carcinoma), cardiovascular diseases (myocardial infarction, cerebral stoke, and sudden cardiac death).[7, 8] Free radicals from smoking and diseases generate the lipid peroxidation process in an organism. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acid peroxidation in the cells. An increase in free radicals causes overproduction of MDA which is commonly known as a marker of oxidative stress[9] In vitro investigations have consistently demonstrated that cigarette smoke depletes plasma of carotenoids and α-tocopherol. The present study was carried out to investigate serum lycopene and α-tocopherol levels in healthy northeast Thai elderly living in Khon Kaen province. Serum MDA which helps predicting of oxidative stress[10], was also determined.

Corresponding Author: Dr Patcharee Boonsiri, Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand
Tel: 66 43 348386; Fax: 66 43 348386
Email: patcha_b@kku.ac.th

Materials and methods

Subjects

The 207 healthy elderly with the age range of 60 to 91 years old, who lived in WangNoi district, Khon Kaen province, Thailand were enrolled in this study. Fasting blood samples were collected during March, 2005. Their plasma glucose, serum uric acid, total cholesterol, high-density lipoprotein-cholesterol, liver function enzymes (aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase), kidney function parameters (blood urea nitrogen and creatinine) were within the normal ranges. Subjects were interviewed by questionnaires about their smoking habit. Blood samples collected from the subjects by venipuncture were centrifuged at 1,000 g for 5 min at room temperature. Serum samples were kept at -20 ^{0}C until analysis.

Lycopene and α-tocopherol determination

Lycopene and α-tocopherol were extracted from serum samples before determination. Serum was mixed briefly with 200 µL of 0.1 mmol/L sodium dodecyl sulphate (SDS) reagent for 1 min, then 200 µL of ethanol containing 50 µmol/L of tocopherol acetate (as internal standard) was added to the sample. n-Heptane containing 0.5 g butylated hydroxytoluene (BHT) per liter was then added, mixed vigorously for 2.5 min and centrifuged at 3,000 g for 15 min to separate the organic phase from the aqueous phase. The 700 µL of heptane layer was evaporated under nitrogen gas at 40 ^{0}C. The residue was reconstituted with 100 µL of freshly prepared mobile phase (acetonitrile:methanol:dichloromethane = 4:4:1). Lycopene and α-tocopherol levels were measured by a reverse-phase HPLC system with a dual wavelength UV-VIS detector (model 2487, Waters). Mobile phase was delivered to a C18 ODS-2 Spherisorb column at a flow rate of 1 mL/min by an isocratic pump (model 600 PUMP, Waters). The column was equilibrated with the mobile phase for 5 column volume. The UV-VIS detector was set at wavelength 292 and 450 nm which are the λmax of standard α-tocopherol and lycopene (Cat. No. T-3251 and L-9879, Sigma Company, respectively).

Malondialdehyde determination

The concentration of MDA based on the reaction with thiobarbituric acid (TBA) was determined according to the modification of previously published method.[11] The 100 µL of serum was mixed with 1.5 mL of 25 nmol/L TBA, 1.5 ml HCl, 550 µL distilled water, 200 µL of 8.1 % SDS and 50 µL of 7.2 % BHT. The reaction mixture was incubated at 90 ^{0}C for 15 min and rapidly cools for 10 min. Afterthat, 0.5 mL of distilled water and 3 mL of n-butanol in pyridine were added to the mixed reaction. They were mixed vigorously and centrifuged at 3000 g for 15 min. The absorbance of MDA-TBA product was measured by a spectrofluorometer at 520 nm of excitation and 550 nm of emission.

Ethics

Ethical approval for the study was obtained from the Ethics Committee of Khon Kaen University (HE 480210) and the subjects gave written informed consent.

Statistical analysis

All results were presented as mean±SE (95% CI).The difference between groups and data correlation were obtained by two sample t-test and Pearson correlation respectively. Using the STATA program ver.7.0, a value of $p<0.05$ was considered statistically significant.

Results

Mean serum lycopene and α-tocopherol levels of 207 healthy Thai elderly (72 males and 135 females) were

Table 1. Serum lycopene and α-tocopherol levels in healthy Thai elderly.

antioxidant	Mean ± SE[1] (95% CI)		*p*-value
	Male (N = 72)	Female (N = 135)	
Lycopene (µmol/L)	0.18 ± 0.02 (0.13 - 0.22)	0.32 ± 0.02 (0.27 - 0.37)	<0.01*
α-tocopherol (µmol/L)	18.96 ± 0.71 (17.53 - 20.39)	23.78 ± 0.74 (22.31 - 25.25)	<0.01*

[1]Values mean ± SE (standard error of mean); * statistically significant ($p<0.01$)

Table 2. Serum antioxidants and oxidative stress marker in current smoking and non-smoking Thai elderly.

Serum	Mean ± SE[1] (95% CI)		*p*-value
	Current smoker (N = 25) (23M/2F)	Non-smoker (N = 182) (49M/133F)	
Lycopene[a] (µmol/L)	0.17 ± 0.02 (0.12 - 0.22)	0.28 ± 0.02 (0.24 - 0.32)	0.04*
α-tocopherol[a] (µmol/L)	20.19 ± 7.45 (17.11 - 23.26)	22.37 ± 8.21 (21.17 - 23.57)	0.21
MDA[b] (µmol/L)	1.55 ± 0.10 (1.33 - 1.77)	1.35 ± 0.41 (1.27 - 1.43)	0.09

[1]Values mean ± SE (standard error of mean); [a]serum antioxidant; [b]oxidative stress marker; *statistically significant ($p<0.05$)

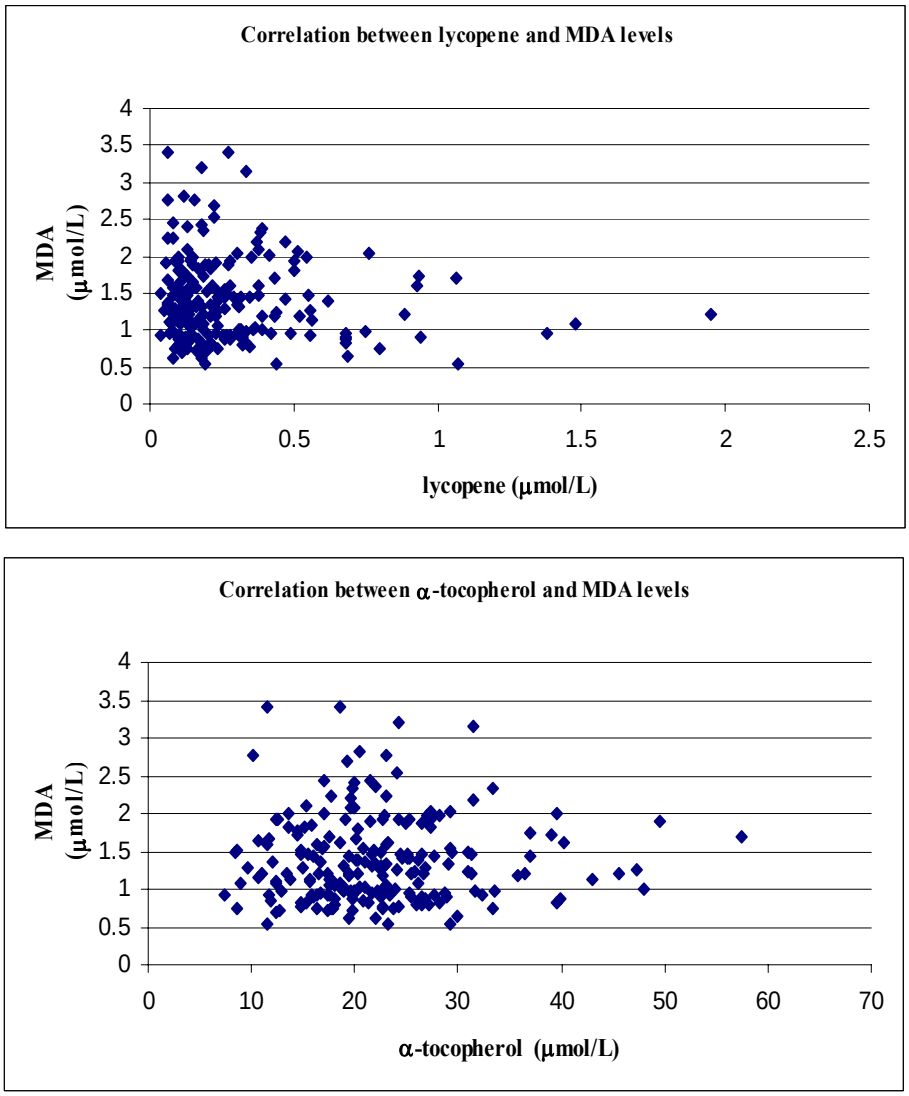

Figure 1. Correlation between (a) lycopene and MDA levels (r=-0.88), (b) α-tocopherol and MDA levels (r=0.20) in healthy Thai elderly.

0.27 µmol/L (95% CI = 0.23-0.31) and 22.10 µmol/L (95% CI = 20.99-23.22) respectively. Lycopene levels in males (0.18 µmol/L, 95% CI = 0.13-0.22) was significantly lower than those in females (0.32 µmol/L, 95% CI = 0.27-0.37) (p<0.01). In addition, α-tocopherol levels in males (18.96 µmol/L, 95% CI = 17.53-20.39) was also significantly lower than those in females (23.78 µmol/L, 95% CI = 22.31-25.25), (p<0.01) (Table 1).

The subjects in this study were classified into two groups according to their smoking behavior; current smoking group (N=25) and non-smoking group (N=182) (Table 2). Current smokers consisted of 23 males (31.94 % of 72 males) and 2 females (1.4 % of 135 females). Lycopene levels in the current smoking (0.17 µmol/L; 95% CI = 0.12-0.22) was significantly lower than non-smoking group (0.28 µmol/L; 95% CI = 0.24-0.32) (p=0.04). It seems that the current smoking group tended to have lower serum α-tocopherol level (20.19 µmol/L 95% CI = 17.11-23.26) than the non-smoking group (22.32 µmol/L 95% CI = 21.17-23.57) but no statistical significance was found (p=0.21). MDA levels in current smokers and non-smokers were 1.55 µmol/L (95% CI = 1.33-1.77) and 1.35 µmol/L (95% CI = 1.27-1.43) respectively. Mean level of MDA in current smokers was higher than non-smokers (p=0.09). Moreover, serum lycopene was inversely related with MDA levels (r=-0.88, p=0.03) whereas α-tocopherol was not related with MDA level (r=0.20, p=0.77) (Fig 1).

Discussion

The levels of the lycopene and α-tocopherol determined reflect serum concentrations before the enrolment of subjects under habitual dietary intakes in WangNoi district, Khon Kaen province, Thailand. Because the leading health problem in elderly Thais were cardiovascular disease and cancers, lycopene and α-tocopherol are possibly involved in the prevention of coronary heart diseases by inhibiting oxidative modification of LDL cholesterol.[12, 13] In this study, both lycopene and α-tocopherol levels in males were significantly lower than in females (p<0.01) (Table 1). Lower levels of lycopene and α-tocopherol in males may have strongly crucial role to maintain the balance oxidative stress in healthy conditions because 31.94% of males are current smokers, whereas 1.4% females are current smokers.

In the present study, the level of lycopene in the current smoking group was significantly lower than in non-smoking group (p=0.04). This was supported by many studies that smokers have lower plasma concentrations of most carotenoids than non-smokers.[14-16] The current smoking group had lower serum α-tocopherol level than non-smoking but not statistically significant (p=0.21)

(Table 2). The possible explanation is that cigarette smoking is well known for introducing a source of free radicals into the body.[6] It contains many oxidants and free radicals, which can increase lipid peroxidation and DNA damaging.[17] Lycopene and α-tocopherol may be used to neutralize the free radicals generated from smoking. The smoker and non-smoker may be differing in the handling of micronutrients.

The current smokers had higher MDA levels than non-smokers. This results suggested that the current smoking group had higher risk of oxidative stress than the non-smoking group. Moreover, serum lycopene in healthy Thai elderly was inversely related with MDA levels (r=-0.88) while α-tocopherol showed no correlation with MDA levels (r=0.20) (Figure 1). There is a report that lycopene is the most potent antioxidant activity among carotenoids.[18] It has 2 and 10 times more singlet oxygen quenching activity than ß-carotene and α-tocopherol, respectively.[19] Metabolite of lycopene may be bioactive and responsible for the beneficial effects because isomers of lycopene may have different biological activities than α-tocopherol.[20] Thus, lycopene may be more effective to scavenge free radical in these Thai elderly who had risk of oxidative stress such as smoking, than α-tocopherol. The results from this study suggest that supplemental dietary antioxidants, especially lycopene from local fruits and vegetables may have a beneficial role in the prevention of chronic diseases at high-risk oxidative stress such as smoking.

Conclusion

Serum lycopene and α-tocopherol baseline levels were obtained in healthy northeast Thai elderly. Males had significant lower lycopene and α-tocopherol levels than females. MDA level was higher in smokers regardless of gender. Serum lycopene was inversely related with MDA level, whereas α-tocopherol showed no correlation with MDA level. Further research should be emphasized on local antioxidant nutrient supplementation for these elderly to help preventing oxidative stress and diseases.

Acknowledgements

This study was supported by Khon Kaen University Research grant, the Faculty of Medicine and Graduate School, Khon Kaen University grants.

References

1. Irshad M, Chaudhuri PS. Oxidant-antioxidant system: role and significance in human body. Indian J Exp Biol 2002;40:1233-1239.
2. Favier A. Oxidative stress in human diseases. Ann Pharm Fr 2006;64:390-396.
3. Tapiero H, Townsend DM, Tew KD. The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother 2004;58:100-110.
4. Bruno RS, Traber MG. Cigarette smoke alters human vitamin E requirements. J Nutr 2005;135:671-674.
5. Trobs M, Renner T, Scherer G, Heller WD, Geiss HC, Wolfram G, Hass GM, Schwandt P. Nutrition, antioxidants, and risk factor profile of nonsmokers, passive smokers and smokers of the Prevention Education Program (PEP) in Nuremberg, Germany. Prev Med 2002;34:600-607.
6. Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. Toxicology 2002;180:121-137.
7. Dautzenberg B. Tobacco-related diseases. Rev Prat 2004;54:1877-1882.
8. Nordquist LT, Simon GR, Cantor A, Alberts WM, Bepler G. Improved survival in never-smokers vs current smokers with primary adenocarcinoma of the lung. Chest 2004;126:347-351.
9. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005;15:316-328.
10. Gawel S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek 2004;57:453-455.
11. Wade CR, van Rij AM. Plasma thiobarbituric acid reactivity: reaction conditions and the role of iron, antioxidants and lipid peroxy radicals on the quantitation of plasma lipid peroxides. Life Sci 1988;43:1085-1093.
12. Rao AV. Lycopene, tomatoes, and the prevention of coronary heart disease. Exp Biol Med (Maywood) 2002;227:908-913.
13. Buijsse B, Feskens EJ, Kromhout D. Nutrition and health--unclear association between intake of vitamin E and the risk of coronary heart disease. Ned Tijdschr Geneeskd 2003;147:2007-2011.
14. Garg N, Singh R, Dixit J, Jain A, Tewari V. Levels of lipid peroxides and antioxidants in smokers and non-smokers. J Periodontal Res 2006;41:405-410.
15. Tanabe N, Toyoshima H, Hayashi S, Miyanishi K, Funazaki T, Obata A, Wakai S, Enoki S, Hashimoto S, Kamimura K. Effects of smoking and drinking habits and vitamin A intake on serum concentrations of beta-carotene and retinol. Nippon Eiseigaku Zasshi 1992;47:679-687.
16. Van Antwerpen VL, Theron AJ, Richards GA, Van Der Merwe CA, Van der Walt R, Anderson R. Relationship between the plasma levels of beta-carotene and lung functions in cigarette smokers. Int J Vitam Nutr Res 1995;65:231-235.
17. Ceylan E, Kocyigit A, Gencer M, Aksoy N, Selek S. Increased DNA damage in patients with chronic obstructive pulmonary disease who had once smoked or been exposed to biomass. Respir Med 2006;100:1270-1276.
18. Weisburger JH. Lycopene and tomato products in health promotion. Exp Biol Med (Maywood) 2002;227:924-927.
19. Heber D, Lu QY. Overview of mechanisms of action of lycopene. Exp Biol Med (Maywood) 2002;227:920-923.
20. Frohlich K, Kaufmann K, Bitsch R, Bohm V. Effects of ingestion of tomatoes, tomato juice and tomato puree on contents of lycopene isomers, tocopherols and ascorbic acid in human plasma as well as on lycopene isomer pattern. Br J Nutr 2006;95:734-741.