Vitamin E and MDA Concentrations in Plasma of Healthy Young Adult, Elderly and Pregnancy

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ABSTRACT

Vitamin E is one of essential micronutrients. It is an antioxidant which potentially protects biomolecules against oxidative damage. One of parameters of oxidative damage is MDA or lipid peroxide. In this study, concentrations of plasma vitamin E as an antioxidant and MDA as one of oxidative damage markers were measured. The subjects consisted of five groups: (I) male medical students (young adult), (II) female medical students (young adult), (III) nursery inhabitants in Yogyakarta province (Abiyoso), (IV) nursery inhabitants in Manado, North Sulawesi Province (Senja Cerah), as the old ages, and (V) pregnant women within third trimester (of Prenatal Care Unit in Sardjito Hospital). The concentrations of Vitamin E (mg/dl) and MDA (nmol/ml) were as follows: Young Adult Male (n= 28) 20,8 ± 3,8 and 0,190 ± 0,057 ; Young adult Female (n=28) 21,9 ± 4,5 and 0,123 ± 0,057 ; Old age Yogyakarta (n=41) 30,6 ± 10,4 and 0,291 ± 0,062 ; Old age Manado (n=40) 26,5 ± 4,5 and 0,391 ± 0,081 ; Pregnant Women (=28) 39,9 ± 11,3 and 0,165 ± 0,067. Groups of people who were hyperlipemic tend to have high concentration of Vitamin E, while group IV who eat less vegetable protein and more PUFA tend to have higher MDA concentration.

Keyword : Malonal dehyde (MDA), plasma, young adult, elderly, pregnancy

INTRODUCTION

Human is one of living organisms that exposed to a certain living period. Among this periods are young adult, old age and pregnancy. During each of that living periods, human is susceptible to nutrients deficiencies. While protein caloric malnutrition still exists in the third world inhabitants, issues of nutritional deficiencies which concerned to several aspects, such as – metabolic function disturbances and imbalance between nutrients – leading to degenerative and chronic diseases are interested (get increased attention). Including to nutritional imbalance, concerned to micronutrients or vitamins and minerals. Vitamin E is one of essential micronutrients. It is an antioxidant wnich potentially protects biomolecule against oxidative damage. Oxidative stress is increasingly recognized as an adverse factor in aseing, and in a large number of chronic diseases such as heart disease, cancer, diabetes and Alzheimer's disease. Included to human vitamin E deficiency symptoms are characterized by a progressive peripheral neuropathy with a specific ' dying back 'of the large caliber axon of the sensory neurons, with results an incoordination and an inability to walk (Sokol 1993, cit Traber, 1998) and disturbed pregnancy (Wang et al. 1991b). Oxidative stress is a shift in imbalance

between cellular oxidants and antioxidants towards the former. The major source of these reactive oxygen species and radicals in the body is the 'leakage' of electrons during oxidative metabolism for conversion of foodstuffs to energy (Traber, 1998).

During certain normal but special living periods, such as old age and pregnancy, production of oxidant is increased. One of parameters of oxidative damage is MDA or lipidperoxide. In this study, concentrations of plasma vitamin E as antioxidant and MDA as one of oxidative damage markers was measured. Many food materials contained Vitamin E. Vitamin E deficiency is quite rare in human, but does occur as a result of genetic abnormalities in alpha-TTP (alpha tocopherol transfer protein) and as a result of various fat malabsorption syndromes. In this study the concentrations of Vitamin E as an antioxidant and MDA as a product of oxidative damage during certain normal but special living periods, such as young adult, old age and pregnant were measured.

MATERIALS AND METHODS

Institutional approval was obtained to conduct this study from Ethical Clearance Committee of Medical Faculty of Gadjah Mada University, and informed consent had been signed by the subjects. Subjects were male (n = 28) and female (n = 28) medical students, as young adult subjects, a nursery inhabitants in Yogyakarta (n = 41) and a nursery inhabitants in Manado (n = 40) as groups of elderly from different part of Indonesia and normal pregnant women at third trimester who visit Antenatal Care unit of Sardjito Hospital, as subjects of pregnant women group. No dietary manipulation was done to all of the subject.Venous blood sample was collected from a total of 165 subjects.

Venous blood samples (4 ml) were collected after overnight fast circa 12 h, from the median cubital vein before 9 AM. As anticoagulant EDTA (1 g/L blood) were used. In addition, (2 g / L) 50 mL of 2000 ppm BHT/ 500 mL sample was added to plasma for those samples stored for measurement of the TBA-MDA adduct (Wander et al. 1996)

Lipid peroxides were determined by the method of Yagi (1982, cit Wang et al.1991a), which measures thiobarbituric acid-reactive products and expressed the data in terms of malondialdehyde (MDA). Four milliliters of 1/12 N sulfuric acid and 0.5 ml of 10% of phosphotungstic acid were added to 20 microliters plasma and mixed thoroughly. After centrifugation at 3000 rpm for 10 minutes, the liquid phase was decanted. Two ml of 1/12 N of sulfuric acid and 0,3 ml of 10 % phosphotungstic acid were added to each sample , mixed , and centrifuged again.The liquid phase was decanted. Four milliliter of double-destilled water added and 1 ml of TBA reagent (0.67 % 2-thiobarbituric acid/ acetic acid, 1:1) were then added to each sample, mixed, and heated at 95° C for one hour. Samples were cooled with tap water. Five milliliter of of n-buthyl-alcohol was added, and the samples were vigorously shaken for1 minute and centrifuged. The n-buthyl-alcohol phase, which contained the lipid peroxides, was used for malondialdehyde analysis with a Shimadzu FR –510 flourospectrophotometer (Kiyoto) with excitation at 515 nm and emission 553 nm. Tetramethoxy propane (Sigma) was used as standard and double-distilled water as control. Recovery of exogeneusly added standard was 98% and the coefficient of variation for the assay was 6.5%. Resulted in assay of varying sample volumes resulted in linear responses parallel to the standard curve.

Vitamin E was determined by fluorometric method. Alpha-tocopherol was measured by fluorometrically as described by Katsui (cit wang et al., 1991a). One milliliter of double distilled water and 1ml ethanol were added to 0.2 ml of serum and mix thoroughly. Five milliliters of n-hexane was added and the samples were vigorously shaken for one minute. Samples were centrifuged at 1000 rpm for 15 minutes and the hexane phase was separated and analysis for alpha-tocopherol, with a Shimadzu FR510 flourospectrophotometer (Kiyoto) with excitation at 295 nm and emission 320 nm. DL-alpha tocopherol (E.Merck, West Germany) was used as standard and double distilled water as control. Recovery of exogenously added vitamin E was

95%, and the coefficient of variation for the assay was 5,5 %. Assay of varying sample volumes resulted in linear responses parallel to the standard curve. All the data were analyzed using analysis of variance and Students t'test (Yamane, 1970).

RESULTS AND DISCUSSION

The concentrations of vitamin E and MDA are summarized in Table 1. Vitamin E concentrations were similar in female young adult and male young adult. But the concentration of MDA of young adult male was higher than in young adult female, although non significant. This result was in agreement with the higher physical activity of the male subjects (Unpublished data). The production of reactive oxygen species is high in people with high physical activity (Evans, 2000). As in the group III and IV the number of male subjects compared to the number of female subjects was too small, and no hormonal different between male and female in the period of menopause, so subjects from the nursery not differentiated between male and female. The vitamin E concentrations of the other three group of subjects significantly higher than the two group of young adult. Vitamin E is associated with apo-B lipoprotein components (Dutta-roy, 1994), so its concentration is related to lipid concentration in that lipoprotein. It is likely that lipids concentration of the elderly tend to be higher than the young subjects (Assmann, 1982), the vitamin E concentration of elderly also tend to be higher than young adult. Vitamin E concentration of the old age group from Manado, is significantly lower than that of Yogyakarta. The diet composition of Manado was different from diet composition of Yogyakarta. The nursery inhabitant in Yogyakarta eats more Vitamin E sourses than the nursery inhabitan in Manado. The nursery inhabitant in Yogyakarta eats less fish than the nursery inhabitant in Manado (Manampiring 2001). The fish are source of polyunsaturated fatty acid. Polyunsaturated fatty acids are substrate of oxidative damage which result malondialdehyde (MDA), so that MDA of elderly from Manado is higher than MDA of elderly from Yogyakarta. The highest Vitamin E concentration was of pregnant women. Normally lipid concentration of pregnant women, especially triacylglycerol was extremely high. This is agree with the character of the pregnant women who have to deliver nutrients to their fetus and to prepare breast feeding for her baby

Table 1. Vitamin E and Malonaldehyde (MDA) Concentrations In Plasma Of Healthy Status In The Periods Of Young Adult, Old And Pregnancy

Group of Subject	N	Vitamin E X ± SD (mg/dl)	MDA X ± SD (nmol/ml)	Remark
Young Adult Male	28	20,8 ± 3,8 *	0,190 ± 0,057*	The different number of star in a column shows that there is a significant different among the means. ($p < 0.05$)
Young Adult Female	28	21,9 ± 4,5 *	0,123 ± 0,057*	
Old age Yogyakarta	41	30,6 ± 10,4 **	0,291 ± 0,062**	
Old age Manado	40	26,5 ± 4,5 ***	0,391 ± 0,081***	
Pregnant Women	41	39,9 ± 11,3****	0,165 ± 0,067*	

(McNamara, 1994). Normally vitamin E in pregnant women is high, and the result of this investigation was the highest and it was agree with the lowest MDA concentration. Arachidonic acid is a substrate for thromboxane and prostacyclin synthesis. Imbalance between thromboxane and prostacyclin in preeclamsia is associated with imbalance between lipid peroxide and vitamin E in maternal blood (Wang et al., 1991b). Therefore the results of this study show that in normal pregnant although lipids concentrations were high, the concentrations of MDA were low it shows that the high vitamin E concentrations in pregnant women protects lipids against oxidation.

CONCLUSION

The blood concentration of vitamin E was influenced by various conditions. Dietary Vitamin E deficiency is rare because many foods contain vitamin E. Possible causes of vitamin E deficiency are malabsorptions of fat, deficiency of apo-B containing lipoproteins and, deficiency of alpha tocopherol binding protein. Apo-B containing lipoproteins and alpha-tocopherol binding protein are needed to transport vitamin E in blood circulation. Concentration of MDA as a product of oxidative damage was influenced by PUFA and vitamin E. PUFA is a substrate of oxidative damage and vitamin E protects PUFA from oxidative damage.

So there was a contradiction in a group of people that their diet contained more PUFA but less Vitamin E. It is well known that PUFA is an anti-atherosclerotic fatty acid but PUFA is substrate of oxidative damage which MDA is the product. MDA is substance that toxic for endothelial cell, which result atherosclerosis. So it is recommended that dietary intake of nutrients has to be balanced.

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