

ANTIOXIDANT VITAMIN POOL IN SENIOR POPULATION

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SUMMARY

Aim: To compare plasma concentration of α -tocopherol and ascorbic acid in healthy seniors (age over 65 years), senior patients with either diabetes mellitus, acute myocardial infarction or dyslipidemia and recommended values of these vitamins. **Methods:** Studied groups included 30 patients with diabetes mellitus (DM); 30 patients 1 – 2 weeks after acute myocardial infarction (AMI); 11 patients with lipid metabolism disorder (LD, total cholesterol > 6.2 mM); and control group of 27 healthy persons. **Results:** Concentration of α -tocopherol in DM group was $14.6 \pm 5.3 \mu\text{M}$, in AMI group $13.7 \pm 5.6 \mu\text{M}$, in LD group $15.9 \pm 5.6 \mu\text{M}$ and in control group $12.9 \pm 4.1 \mu\text{M}$. No statistically significant differences were found. However, comparison of determined values with levels recommended for prevention revealed remarkable low plasma concentration of α -tocopherol in the Czech population. Plasma concentration of ascorbic acid in DM group was $47.07 \pm 22.80 \mu\text{M}$, in AMI group $33.15 \pm 12.81 \mu\text{M}$, in LD group $45.59 \pm 23.02 \mu\text{M}$ and in control group $43.28 \pm 26.57 \mu\text{M}$. No statistically significant differences were found between the controls and individual groups of patients. Plasma concentrations of vitamin C reached the recommended value in all cases except the AMI group, where it was significantly lower. **Conclusion:** Seniors in the Czech population were proved to be significantly short of α -tocopherol, minor shortage of vitamin C was found only in group of patients with myocardial infarction.

Key words: vitamin E, vitamin C, antioxidants, seniors

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INTRODUCTION

Ischemic heart disease as well as diabetes mellitus is accompanied by increased oxidative stress, which plays an important role in the pathogenesis of vascular complications. Oxidative stress occurs

when the reactive oxygen species (ROS) evolution overcomes the capacity of antioxidative system and the balance between their production and elimination becomes impaired. Antioxidative system comprises of both endogenous part – antioxidative enzymes and exogenous part – especially vitamins E, C, β -carotene.

Vitamin E (α -tocopherol), the major lipid soluble antioxidant, occurs in plasma especially in VLDL and LDL fractions, where it exhibits important protective role by preventing lipid peroxidation. In tissues α -tocopherol protects polyunsaturated fatty acids of membrane phospholipids. The aqueous phase of plasma contains a wide range of molecules with antioxidant properties. One of these is ascorbic acid, a very good scavenger of several radicals, which is able to regenerate α -tocopheryl radicals in membranes or lipoproteins. Antioxidative function is exhibited also by other endogenous compounds, especially by uric acid, glutathione or albumine.

Dietary intake may affect predominantly the levels of antioxidative vitamins. Some epidemiologic studies have shown a negative correlation between intake of fruits and vegetables and mortality from ischemic heart disease. Insufficient supply of food with balanced antioxidative composition and simultaneous increase of the risk of ischemic heart disease are characteristic especially for senior population. For this reason our study was aimed at comparison of plasma concentration of α -tocopherol and vitamin C in senior patients (over 65 years) with diabetes mellitus, acute myocardial infarction, lipid metabolism disorder and control group to check whether potential shortage of these vitamins might be connected with manifestation of the above mentioned disorders. We also compared the levels detected in Pardubice population with those recommended (1) for cardiovascular disease and cancer prevention in Germany, Austria and Switzerland.

PATIENTS AND METHODS

The study included 98 subjects fulfilling the following criteria:

- 30 patients with diabetes mellitus treated by insulin therapy (DM, average age 72.7 years);
- 30 patients 3 days – 2 weeks after acute myocardial infarction (AMI, average age 73.1 years);
- 11 patients with lipid metabolism disorder (LD, total cholesterol > 6.2 mM, average age 75.7 years);
- control group of 27 persons without manifestation of cardiovascular disease or another disease connected with increased production of reactive oxygen species, total cholesterol < 5.6 mM (average age 77.6 years).

A written informed consent was obtained from all the participants before starting the protocol and the study was approved by Hospital Ethical Committee on Human Research.

Peripheral venous blood samples were obtained. Freshly frozen plasma samples were stored at -80°C . To 300 μl plasma for vitamin C estimation 10 μl 100 mM dithioerythritol (antioxidant) was added.

Plasma concentration of α -tocopherol was determined with HPLC (Shimadzu, Japan; Biospher Si C_{18} column, 120 PSI, MAC

250 x 4 mm, particle size 5 μm) using the mixture of acetonitrile - hexan - methanol (40:15:45, v/v/v). The absorbance of α -tocopherol was detected at 292 nm. As internal standard tocopherol acetate was used.

Plasma concentration of ascorbic acid was determined by ion-pair HPLC with coulometric detection. After protein precipitation (10% trichloroacetic acid/10 mM oxalic acid and extraction with ethyl ether) the samples were subjected to HPLC (Discovery- C_{18} column 150 x 4 mm, particle size 5 μm , Supelco, flow rate 0.5 ml/min). The mobile phase consisted of 100 mM sodium dihydrogen phosphate and 100 mM sodium acetate pH = 5.4, 400 μM dodecyltrimethylammonium chloride, 75 μM tetraoctylammonium bromide, 15% methanol. Vitamin C was quantified with ESA Coulochem II 5100 A electrochemical detector (screening potential of electrode 1 and electrode 2 was -200 mV and $+300$ mV, respectively, gain range was 5 μA). Statistical method: t-test, ADSTAT.

RESULTS AND DISCUSSION

Plasma concentrations of α -tocopherol in senior patients and the corresponding control group are shown in Table 1. As α -tocopherol is a lipophilic vitamin, its values have been often standardized to the lipid content. Standardization used to be performed per content of triacylglycerols, per cholesterol content or per the sum of lipids (2). As only total cholesterol was determined for all the studied subjects, correlation analysis between the α -tocopherol and cholesterol was done. Statistically significant correlation was found only in case of the control group. For this reason we used in our data evaluation directly the plasma concentrations of α -tocopherol. No statistically significant difference was found among four groups of seniors compared. Thus the presumption, that oxidative stress of various intensity, which is involved in pathogenetic mechanism of studied disorders, could be reflected in differences in vitamin E level, was not confirmed. This presumption was based on several studies, which found correlation between higher dietary intake or plasma concentration of antioxidant vitamins and lower occurrence of ischemic heart disease (3, 4, 5), as well as on the reports on lower levels of antioxidant vitamins in the serum of diabetic patients (6). The lack of correlation found in our study indicates that the multiple factors are involved in re-establishment of the balance between ROS evolution and their elimination.

Plasma concentrations of ascorbic acid in senior patients and the control group are shown in Table 2. The level of this vitamin was remarkably low in the group with acute myocardial infarction, even if the difference between these patients and the control group did not reach the statistical significance (probably due to the large

Table 1. α -Tocopherol concentration in studied groups

Groups of patients	Plasma concentration of α -tocopherol
DM group	$14.62 \pm 4.92 \mu\text{M}$
AMI group	$14.54 \pm 5.48 \mu\text{M}$
LD group	$15.50 \pm 5.86 \mu\text{M}$
Control group	$13.39 \pm 4.23 \mu\text{M}$

Table 2. Vitamin C concentration in studied groups

Groups of patients	Plasma concentration of ascorbic acid
DM group	$47.07 \pm 22.80 \mu\text{M}$
AMI group	$33.15 \pm 12.81 \mu\text{M}$
LD group	$45.59 \pm 23.02 \mu\text{M}$
Control group	$43.28 \pm 26.37 \mu\text{M}$

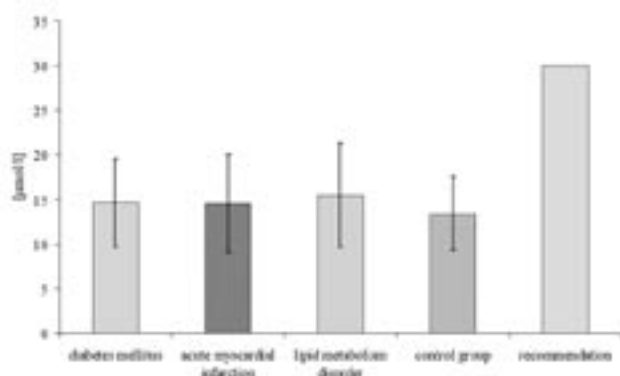


Fig. 1. The comparison of plasma α -tocopherol levels in individual groups of patients and the control group with the value recommended for prevention of cardiovascular disease.

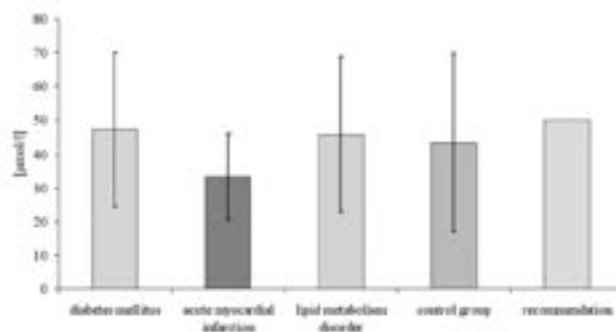


Fig. 2. The comparison of plasma vitamin C levels in individual groups of patients and the control group with the value recommended for prevention of cardiovascular disease.

variance within the control group). Low level of ascorbic acid in patients with AMI is in accordance with the results of Gey (7), who found negative correlation of this vitamin level with the risk of ischemic heart disease and confirms the positive effect of vitamin C on the diminishing of the atherosclerosis development.

Further we compared α -tocopherol and vitamin C levels determined in senior Czech population with values recommended for cardiovascular disease and cancer prevention by German, Swiss and Austrian Society for Nutrition (1). As it can be seen from Fig. 1, remarkable shortage of α -tocopherol was found in the elderly Czech population, in all tested groups. This is in accordance with previously published data on adult Pardubice population (8). Insufficient intake of vitamin E was reported in children population as well (9).

The mean values of vitamin C in elderly population do not reach the recommended level, but they are close to that, with only exception of patients with myocardial infarction (Fig. 2).

The antioxidant vitamin deficiency seems to be important risk factor especially in the Czech population, due to the high morbidity and mortality of cardiovascular diseases. In spite of the fact that the Czech dietary habits have been changing in a positive way since 1989, the low intake of vegetables and fruits and another sources of antioxidant vitamins, such as vegetable oils, still seems to be an indispensable factor in coronary heart disease, which, according to our findings, endangers especially senior population.

CONCLUSION

Between senior patients with diabetes mellitus, acute myocardial infarction, hypercholesterolemia and the corresponding control group no significant differences in α -tocopherol levels were found. In case of vitamin C consistently lower level of this vitamin was detected in the group of patients with myocardial infarction. Our study has revealed that senior population of Pardubice was proved

to be highly significantly short of α -tocopherol in comparison with recommended level.

Acknowledgements

The authors are very grateful to MUDr. I. Bureš, Assoc. prof. MUDr. J. Novák, CSc., MUDr. B. Kašková and MUDr. J. Pavlíčková from Nemocnice Pardubice, who participated in the selection of patients. The study was supported by the Ministry of Education, Youth and Sports of the Czech Republic grant No. 253100002 and by the Grant Agency of the Czech Republic grant No. 203/02/0023.

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