Journal of Applied Sciences

ISSN 1812-5654

science alert

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Journal of Applied Sciences 7 (13): 1795-1799, 2007
ISSN 1812-5654

Age-Related Alterations of Plasma Lipid Peroxidation and Erythrocyte Superoxide Dismutase Activity in Different Ethnic Groups of Gorgan

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Abstract: Free radicals have been proposed as important causative agents of ageing. The free radical theory of ageing postulates that ageing is caused by free radical reactions. These highly reactive species can cause oxidative damage in the cell. The purposive of this study was to investigate the alteration in plasma lipid peroxidation and erythrocyte superoxide dismutase activity in 2 different ethnic groups of Fars and Turkmen healthy people. We measured plasma lipid peroxidation levels (lipid peroxidation expressed as malondialdehyde) and erythrocyte superoxide dismutase activity. Study include 350 (175 Fars and 175 Turkmen male) apparently healthy individuals. Erythrocyte superoxide dismutase activities were determined in 2 different ethnic groups of Fars and Turkmen consisting of healthy individuals between 26-60 years of age {26-30 (n = 30), 3-35 (n = 30), 36-40 (n = 30), 41-45 (n = 30), 46-50 (n = 25), 51-55 (n = 15) and 56-60 (n = 15)}, respectively. The data was analyzed by Student' t-test. Erythrocyte superoxide dismutase and plasma lipid peroxidation levels in Fars and Turkmen people with 41-45 ages (group 4) and 36-40 ages (group 3) were significantly lower and higher than in the other age groups (Fars groups 1, 2 and 3, Turkmen groups 1, 2), respectively ($p<0.05$). There were no significant relation between the age group 4 (Fars people) and the age groups 5, 6 and 7 ($p>0.05$). There were no significant relation between the age groups 3 (Turkmen people) and the age groups 4, 5, 6 and 7 ($p>0.05$). We found age-related differences in erythrocyte superoxide dismutase activity and plasma lipid peroxidation levels. The results indicate that the balance between antioxidant and prooxidant factors in free radical metabolism shifts towards increased lipid peroxidation with advancing age in 2 ethnic groups. This situation maybe begin in Turkmen people earlier than Fars people. The ethnic origin, diet, heavy working and life style factors of the two populations may explain this differences. Therefore we propose that older Fars and Turkmen people may have elevated requirement for antioxidants. Supplementation with vitamin or dietary free radical scavengers such as vitamin E and C or foodstuff containing these such as tomatoes, oranges and similars have a potential role in boosting antioxidant related defenses and may be important for older people of two ethnic groups.

Key words: Ageing, lipid peroxidation, superoxide, dismutase, ethnic groups

INTRODUCTION

One of the most popular theories of ageing is the free radicals theory of ageing. Aging affects organs and tissues of a given organism in different ways, resulting in different rates of functional decline, all combining to diminish overall ability to meet increased demand, for example in a stress situation (Gagliano *et al*., 2002). More and more health science researchers have concluded that free radicals caused oxidation is centeral process of ageing, and at the heart of age related deterioration. It has been said that ageing is in fact a process of oxidizing or rusting. Ageing results from an accumulation of changes caused by reactions in the body initiated by highly reactive molecules known as free radicals. The changes induced by free radicals are believed to be a major cause of ageing, disease development or death (Harman, 1955). The ageing process produces changes at an apparently unalterable, exponentially increasing rate with advancing age. These changes are small are early in life but rapidly increase with age due to the exponential nature of process (Harman, 1982). Ageing is an inevitable biological process, leading to loss of function and of resistance to stress. Oxidative stress, an unavoidable consequence of

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oxygen metabolism in aerobic cells, is postulated to be one of the most important causes of age related changes (Kokoszka *et al*., 2001). The imbalance between protective antioxidans (antioxidant defence) and increased free radical production, leading to oxidation damage, is known as oxidative stress. Oxidative stress is caused by reactive overload of oxidants, i.e., reactive oxygen species. This impairs cellular functions and contributes to the pathophysiology of many diseases. Reactive oxygen species such as superoxide anions, hydrogen peroxide and hydroxy radicals are produced by both normal aerobic metabolism and environmental protein and membrane lipids (Ross *et al*., 2000; Smirnova *et al*., 2000). An excess production of free radicals is harmful to cells. To cope with the free radicals, animal and human cells express an array of antioxidant enzymes, including superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase. Superoxide dismutase (SOD) play an important role in the protection of cells against the deleterious effect of free radicals by convertising superoxide anions to hydrogen peroxide, which is then transformed to water by glutathione peroxidase or by catalase (Cadet *et al*., 1993). The activities of this antioxidant enzymes in blood are altered in the ageing process (Tatton and Olanow, 1999). In a study where the fluctuation of superoxide dismutase activity in serum and erythrocyte investigated, it was shown that the activity of this enzyme either reduced by ageing (Guemouri *et al*., 1991) or was not change (Sozmen *et al*., 1993). The free radicals are continously produced by hemoglobin, on the result of auto-oxidation, therefore the red blood cells are constantly exposed to the oxidative stress, but the superoxide dismutase is an enzyme that eliminating such oxidant in red blood cells. For this reason the red blood cells is a suitable environment for the study of superoxide dismutase activity. In recent years, much attention has been paid to the role of radicals and antioxidants play in ageing. Erythrocyte enzyme and plasma lipid peroxidation have not studied in this area with respect to age and ethnic groups (Fars and Turkmen) in healthy subjects. For this reasons the present study was designed to determine the changes of plasma lipid peroxidation levels (expressed as malondialdehyde) and erythrocyte superoxide dismutase activity in healthy people of different age and ethnic groups in city of Gorgan.

MATERIALS AND METHODS

Samples were obtained in randomized fashion from 350 (175 Fars and 175 Turkmen male) healthy subjects (between 26-60 years of age {26-30 (n = 30), 31-35 (n = 30), 36-40 (n = 30), 41-45 (n = 30), 46-50 (n = 25), 51-55 (n = 15), 56-60 (n = 15)}. They were chosen from the people refereed to the Department of Biochemistry, Faculty of Medicine, in Gorgan University of Medical Sciences (2005). Healthy people were defined as not having a major medical illness (with doing biochemical tests and control of them by internal specialist doctor), no hospital admissions, no current medication, no physical activity, no smoking and a subjective perception of good health as determined by health questionnaire. None of the subjects received any medical (vitamin E, C) supplement and non-medical antioxidants (tomato, orange, etc.) for one month. Body Mass Index (BMI) was the same in 2 ethnic groups. Blood samples were obtained after an overnight fast in a heparinized tubes. Plasma was separated soon after blood was taken. The plasma malondialdehyde (the level of lipid peroxidation expressed as Malondialdehyde (MDA) and erythrocyte Super Oxide Dismutase (SOD) were determined using laboratory kits and spectrophotometry technique (model JENWAY 6105 UV/VIS) in the laboratory of biochemistry (Faculty of Medicine). Plasma MDA and erythrocyte SOD were determined with Kei Satoh (1978) and Woolliams *et al*. (1983) methods, respectively. The findings were given to software SPSS-10 and statistical analysis was determined by student's t-test. $p < 0.05$ was considered significant.

Malondialdehyde measurement: To 0.5 mL plasma, 2.5 mL of trichloroacetic acid is added and the tube is left to stand for 10 min at room temperature. After centrifugation at 3500 rev min^{-1} for 10 min, the supernatant is decanted and the precipitate is washed once with sulfuric acid. Then 2.5 mL sulfuric acid and 3 mL Thiobarbituric Acid (TBA) in sodium sulfate are added to this precipitate and the coupling of lipid peroxide with TBA is carried out by heating in a boiling water bath for 30 min. After cooling in a cold water , the resulting chromogen is extracted with 4 mL of n-butyl alcohol by vigorous shaking. Separation of the organic phase is facilitated by centrifugation at 3000 rev min^{-1} for 10 min and its absorbance is determined at the wavelength of 530 nm.

Superoxide dismutase measurement: This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride(I. N. T.) to form a red formazan dye. The Super Oxide Dismutase(SOD) activity is then measured (at 505 nm) by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Table 1: Age-related alteration of plasma lipid peroxidation levels and erythrocyte superoxide dismutase activity in 7 different age of Fars groups

Groups	Age (years)	No. of subjects (n)	Mean of age (years)	BMI (kg m^{-2})	SOD activity (U g^{-1} Hb)	MDA levels (nmol mL^{-1})
1	26-30	30	27. 10±1.68	22.96±0.81	1260.10±3.18	2.94±0.49
2	31-35	30	33.26±1.25	23.21±0.83	1259.10±3.18	3.01±0.39
3	36-40	30	37.76±1.33	24.02±0.48	1258.66±2.66	3.07±0.40
4	41-45	30	43.63±1.15	25.05±0.92	932.63±15.16*	5.77±0.29**
5	46-50	25	48.00±1.32	25.20±0.65	931.68±16.74	5.90±0.25
6	51-55	15	53.40±1.29	25.42±0.63	930.20±25.07	5.92±0.35
7	56-60	15	56.93±1.27	25.87±0.67	929.73±12.95	5.95±0. 49

*P<0.05 compared to the age groups 1, 2 and 3, **p<0.05 compared to the age groups 1, 2 and 3, p>0.05 the age group 4 compared to the age groups 5, 6 and 7, p>0.05 compared the age groups 1, 2 and 3

Table 2: Age- related alteration of plasma lipid peroxidation levels and erythrocyte superoxide dismutase activity in 7 different age of Turkmen groups

Groups	Age (years)	No. of subjects (n)	Mean of age (years)	BMI (kg m^{-2})	SOD activity (U g^{-1} Hb)	MDA levels (nmol mL^{-1})
1	26-30	30	27.22±1.43	23.10± 0.61	1233.90±5.78	3.01±0.47
2	31-35	30	33.83±1.11	23.41±0.47	1232.26±5.88	3.07±0.37
3	36-40	30	37.90±1.42	24.13±0.39	835.960±24.91*	5.88±0.33**
4	41-45	30	43.16±1.2	25.23±0.80	833.560±30.0	5.90±0.28
5	46-50	25	48.32±1.18	25.43±0.53	829.480±26.78	5.91±0.27
6	51-55	15	53.00±1.19	25.68±0.55	826.000±25.61	5.92±0.25
7	56-60	15	57.13±1.24	25.97±0.55	824.330±24.16	6.04±0.24

*p<0.05 compared to the age groups 1 and 2, ** p<0.05 compared to the age groups 1 and 2, p>0.05 the age group 3 compared to the age groups 4, 5, 6 and 7, p>0.05 compared the age groups 1 and 2

RESULTS

In present study we determined the plasma levels of malondialdehyde and erythrocyte superoxide dismutase activity in different age and ethnic groups of healthy individuals. The mean MDA and SOD levels were compared in 7 different age and 2 ethnic groups. Values varied significantly with age and ethnic groups, as shown in Table 1 and 2. The mean of MDA levels in the 41-45 age of Fars group (group 4) was significantly higher than in the age groups 1, 2 and 3 ($p<0.05$). Superoxide dismutase activity in the 41-45 age of Fars groups was significantly lower than in the age groups 1, 2 and 3 ($p<0.05$). No significant relation was observed between the age groups 1, 2 and 3 ($p>0.05$).

There was also no significant relation between the age group 4 and the age groups 5, 6 and 7 ($p>0.05$, Table 1).

The mean of MDA levels in the 36-40 age of Turkmen group (group 3) was significantly higher than in the age groups 1 and 2 ($p<0.05$). SOD activity in the 36-40 age of Turkmen groups was significantly lower than in the age groups 1 and 2 ($p<0.05$). No significant relation was observed between the age groups 1 and 2 ($p>0.05$). There was also no significant relation between the age group 3 and the age groups 4, 5, 6 and 7 (Table 2).

DISCUSSION

The ageing process is now the major risk factor for disease and death after about age 28 in the developed countries (Hiramatsu *et al*., 1992). Although free radicals are very likely to contribute considerably to the development of stochastic disorders observed during the process of ageing, the data available so far do not allow a definite answer to the question about whether free radicals do contribute to the initiation and/or propagation of ageing (Sohal and Orr, 1992). Many investigators have studied age and ethnic groups related changes in antioxidant defenses, but the results are controversial (Rikans and Hornbrook, 1997; Ito *et al*., 1999). The role of free radicals and oxidant injury has been repeatedly described in various diseases but rarely healthy people so this article first examines plasma MDA levels and erythrocyte SOD activities on healthy subjects with different age and ethnic groups and shows significance in alteration. An important advantage of this study when compared with those already published was the number of different age groups. Another important point is that our samples come from a general population. The results of this study show that plasma lipid peroxidation (expressed as malondialdehyde) and erythrocyte superoxide dismutase (SOD) activity undergo significant alterations during ageing in 2 ethnic groups. This study shows that plasma lipid peroxidation and erythrocyte SOD activity were significantly increased and decreased in 41-45 age of Fars groups (group 4) and 36-40 age of Turkmen groups (group 3) when compared with the age groups 1, 2 and 3 (Fars groups) and the age groups 1 and 2 (Turkmen groups), respectively. This study also shows that there was no significant relation between the age group 4 (Fars group) and the age groups 5, 6 and 7 and also there was no significant relation between the age group 3 (Turkmen group) and the age groups 4, 5, 6 and 7. Some of the previous studies described that plasma lipid peroxidation and erythrocyte SOD activity increased (Ozturk and Gumuslu, 2004) and some others show increased lipid peroxidation and decreased SOD activity

(Bhagwat, 1997; Inal *et al*., 2001; Ozbay and Dulger, 2002; Guemouri *et al*., 1991), whereas others show no significant differences of age related alteration of SOD activity (Ceballos *et al*., 1992; Ripalda *et al*., 1989). The result of this study are in agreement with the results of studies showing that plasma lipid peroxidation significantly increased with ageing (Ito *et al*., 1999; Ozturk and Gumuslu, 2004; Bhagwat, 1997; Inal *et al*., 2001). Possible sources of elevated free radicals in subjects include increased production of radical oxygen species, especially from lipid peroxidation processes and decreased antioxidant defense systems with ageing (Giugliano *et al*., 1995). Ageing can thus be viewed as a process of irreversible injuries associated with accumulation of these oxidative changes. Erythrocytes are exposed to continuous oxidative stress due to oxygen radicals generated by the auto-oxidation of hemoglobin. Because cellular membranes house the production apparatus of these radicals and because membranes suffer great damage from these radicals, modification of membrane lipids has been proposed to play a major role in the process of ageing (Rikans and Hornbrook, 1997). The ethnic origin, diet, heavy working and life style factors of the two populations may explain this differences. The results of this study are also in agreement with the results of studies showing that erythrocyte SOD activity significantly decreased with ageing in two ethnic groups (Bhagwat, 1997; Inal *et al*., 2001; Ozbay and Dulger, 2002; Ito *et al*., 1999). Possible explanations for our results include reduced antioxidant protection and/or greatly increased amount of free radical with alterations of age that overwhelm the defense system. Other explanations include decreased activity of SOD related to increased free radical production causing oxidation followed by denaturing of the enzyme (Hunt and Wolff, 1991). Superoxide dismutase plays an important role in the detoxification of oxygen-derived radicals, the age-related decrease and increase in SOD activity and lipid peroxidation respectively may lead to an increase vulnerability of erythrocytes from old people to free radical damage. The age dependent decrease maybe due to a progressive enzyme inactivitation by oxidative stress products (inhibition of superoxide dismutase activity by H_2O_2). Many factors such as diabetes mellitus and cancer affect the antioxidant enzyme activities in human begins (Godin *et al*., 1998; Salnikova and Musatova, 1990; Gonzales *et al*., 1984; Marklund *et al*., 1982). These results might overall represent a situation in which the Fars and Turkmen people in 41-45 ages (group 4) and 36-40 ages (group 3) have not evolved a sort of free radicals, antioxidant equilibrium and mechanism of successful ageing. The results indicate that the balance between antioxidant and prooxidant factors in free radical metabolism shifts towards increased lipid peroxidation with advancing age. Finally, we concluded that there were exact relations between MDA and SOD levels and age and ethnic groups in healthy subjects. We think that blood MDA and SOD levels or one of them seem to be effected by age in healthy ethnic groups. We propose that older Fars and Turkmen people may have supraphysiological antioxidants requirement. Supplementation with free radical scavengers such as vitamins E and C or food staff containing these such as tomatoes, oranges and similars have the potential to boost antioxidant defenses and thus may be important for older people of two ethnic groups.

ACKNOWLEDGMENT

I thank to research deputy of Golestan University of Medical Sciences for financial support and helping me in collecting the materials.

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