Age–related alterations of plasma lipid peroxidation and erythrocyte superoxide dismutase activity in different age groups of Gorgan City, Iran

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One of the most popular theories of ageing is the free radicals theory. Ageing results from 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.[1] Ageing is an inevitable biological process, leading to loss of function and of resistance to stress. The imbalance between protective antioxidants (antioxidant defense) and increased free radical production, leading to oxidation damage, is known as oxidative stress.[2] 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) plays an important role in the protection of cells against the deleterious effect of free radicals by converting superoxide anions to hydrogen peroxide, which is then transformed to water by glutathione peroxidase or by catalase.[3] In a study where the fluctuation of SOD activity in serum and erythrocyte was investigated, it was shown that the activity of this enzyme either was reduced by ageing,[4] or was not changed.[5] The free radicals are continuously produced by hemoglobin, as a result of auto-oxidation, therefore, the red blood cells are constantly exposed to the oxidative stress, but the SOD is an enzyme that eliminates such oxidants in red blood cells. For this reason, the red blood cell is a suitable environment for the study of SOD activity. The present study was designed to determine the changes of plasma lipid peroxidation levels (expressed as malondialdehyde [MDA]) and erythrocyte SOD activity in healthy people of different age groups in city of Gorgan, Iran.

Samples were obtained in randomized fashion from 175 healthy subjects between 26-60 years of age (**Table 1**). They were chosen from the people referred to the Department of Biochemistry, Faculty of Medicine, in Gorgan University of Medical Sciences, Gorgan, Iran. Healthy people were defined as not having a major medical illness, no hospital admissions, no current medication, 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, and so forth). Blood samples were obtained after an overnight fast in heparinized tubes. Plasma was separated soon after blood was taken. The plasma MDA (the level of lipid peroxidation expressed as MDA and erythrocyte SOD were determined using commercial kits and spectrophotometry technique (model JENWAY 6105 UV/VIS) in the laboratory of Biochemistry (Faculty of Medicine). The statistical software SPSS version 10 was used, and statistical analysis was determined by students' t-test. A $p<0.05$ was considered significant.

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

Groups	Age (years)	n	Mean age (years)	SOD activity (U/ gr Hb)	MDA levels (nmol/ml)
1	26-30	30	27.1 ± 1.68	1260.1 ± 3.18	2.94 ± 0.49
2	31-35	30	33.26 ± 1.25	1259.1 ± 3.18	3.01 ± 0.39
3	36-40	30	37.76 ± 1.33	1258.66 ± 2.66	3.07 ± 0.4
4	41-45	30	43.63 ± 1.15	932.63 ± 15.16*	5.77 ± 0.29**
5	46-50	25	48 ± 1.32	931.68 ± 16.74	5.9 ± 0.25
6	51-55	15	53.4 ± 1.29	930.2 ± 25.07	5.92 ± 0.35
7	56-60	15	56.93 ± 1.27	929.73 ± 12.95	5.95 ± 0.49

SOD - superoxide dismutase, MDA - malondialdehyde, Hb - hemoglobin.
*$p<0.05$ 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.

Many investigators have studied age related changes in antioxidant defenses, but the results are controversial. The role of free radicals and oxidant injury has been repeatedly described in various diseases, but rarely in healthy people, so this article first examines plasma MDA levels and erythrocyte SOD activities on healthy subjects with 7 different age 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 MDA) and erythrocyte SOD activity undergo significant alterations during ageing. This study shows that plasma lipid peroxidation and erythrocyte SOD activity were significantly increased and decreased in the 41-45 age groups (group 4) when compared with the age groups 1, 2 and 3. This study also shows that there was no significant relation between the age group 4 and the age groups 5, 6 and 7. Some of the previous studies described that plasma lipid peroxidation and erythrocyte SOD activity increased,[6] while others show increased lipid peroxidation and decreased SOD activity,[4] and others show no significant differences of age related alteration of SOD activity.[5] The results of this study are in agreement with the results of studies showing that plasma lipid peroxidation significantly increased with ageing.[4] 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. 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 as membranes suffer great damage from these radicals, modification of membrane lipids has been proposed to play a major role in the process of ageing. The results of this study are also in agreement with the results of studies showing that erythrocyte SOD activity significantly decreased with ageing.[4] Possible explanations for our results include reduced antioxidant protection or greatly increased amounts of free radicals 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. 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, may lead to an increased vulnerability of erythrocytes from older people to free radical damage. These results might overall represent a situation in which the people in the 41-45 age group (group 4) 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 in healthy subjects. We think that blood either MDA or SOD levels, or both seem to be effected by age in healthy individuals. We propose that older people may have supraphysiological antioxidant requirements. Supplementation with free radical scavengers such as vitamins E and C or foodstuffs containing these such as tomatoes and oranges have the potential to boost antioxidant defenses and thus may be important for older people.

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