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# Age-related changes in antioxidant enzyme activities

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## Abstract

Human antioxidant enzymes play a crucial role in cellular defence and protect the cell against ROS. While oxygen metabolism is essential to life on the one hand, it has also long-term toxic effects. The decreasing of enzyme activities is known to be along with aging. The aim of this study was to evaluate the changes in the enzyme activities due to aging, as well as the effect of oxygen free radicals.

In this study, we evaluated age-related changes of erythrocyte Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPX) activities and malondialdehyde (MDA) and glutathione (GSH) levels in healthy subjects. 84 healthy subjects were divided into four groups: 2-11, 12-24, 25-40 and 41-69 years of age.

There were no statistically significant differences in the activities of SOD and GPX enzymes and in the GSH levels among the groups. A statistically significant difference in CAT activity was found between the groups 12-24 and 25-40 ($P<0.05$), but no statistically significant difference was observed between other groups. When the MDA levels of groups 12-24, 25-40 and 41-69 were compared to group 2-11, a statistically significant difference was found ($P<0.001$), but when groups 12-24, 25-40 and 41-69 were compared to each other, no statistically significant difference was found.

Our results show that CAT and SOD activities and GSH and MDA levels are affected in aging. Therefore, we suggest that lipid peroxidation may have a role in the pathophysiological alterations of aging.

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## Introduction

Progressive decline in biochemical and physiological functions of various tissues and organs in an individual is effective in characterisation of aging. Clear data have not yet been existed in related literature, but many studies have reported oxidative stress, disturbances in the functions of energy metabolism and a primary dysregulation of the immune system might play an important role [1,2]. Reactive Oxygen Species (ROS) which are responsible for the damage to a variety of cellular compound [3]. Dismutation of $H_2O_2$, it is removal by Catalase (CAT) and Glutathione peroxidase (GPX) can be effective in the prevention of biological damage caused by ROS. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form such complex series of compounds as reactive carbonyl compounds. There are the most abundant Malondialdehyde (MDA), Superoxide dismutase (SOD) and GPX in cells. Therefore, in the measurement of MDA, SOD and GPX is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both human and model systems [4-10]. In this study, we examined the changes of SOD, CAT and GPX activities and the plasma levels of GSH and MDA in the age-related groups.

## Materials and methods

This study examined 84 healthy subjects (44 male and 40 female) as dependent sex and age attending Cukurova University Hospital. Subjects provided informed consent at the beginning of the study and the study protocol was approved by the Ethics Committee of Cukurova University Hospital. 84 healthy subjects were categorized into four groups: Group 1 (n=20; 2-11 years old, 10 female and 10 male), Group 2 (n=23; 12-24 years old, 10 female and 13 male), Group 3 (n=20; 24-40 years old, 10 female and 10 male) and Group 4 (n=21; 41-69 years old, 10 female and 11 male). Blood samples were collected into heparinized tubes. Samples were centrifuged at 1500 g for 5-10 min and plasma divorced. MDA levels were determined in plasma samples. After divorcing the plasma, erythrocytes were washed three times with saline and erythrocyte packets prepared. Erythrocyte hemolysates were prepared and stored at -25°C for measurement of SOD, GPX, CAT and GSH. Lipid peroxidation was assessed by measuring MDA, an end-product of fatty acid peroxidation according to the method of Sushil JK [11]. SOD activity was determined as reported by Sun et al [12]. The catalase activity was measured by the method of Lartillot et al [13]. GPX activities and GSH levels were determined with the method reported by Beutler E [14].

### Statistical analysis

Data analysis was performed using the computer software Statistical Package for Social Sciences (SPSS; SPSS, Inc., Chicago, IL, USA) for Windows (version 10.0). Continues variables are presented as the mean (standard deviation, SD) or median (min-max) for abnormal distributions and categorical variables are presented as frequencies (%). Comparisons in the distributions of enzyme activities between the groups were evaluated using Mann-Whitney U test, Independent Samples T test and Kruskal-Wallis test for continuous variables (each when adequate) depending on their Gaussian distribution. All tests were two-sided and P-value <0.05 was considered significant.

## Results

In this study, no statistically significant difference in SOD enzyme activities, GPX enzyme activities or GSH levels were found among the groups. A statistically significant difference in CAT activity was found between groups 12-24 and 25-40 (P<0.05), but no statistically significant difference was observed between other groups. When MDA levels of groups 12-24, 25-40 and 41-69 were compared to group 2-11, a statistically significant difference was found (P<0.0001), but when groups 12-24, 25-40 and 41-69 were compared to each other, no statistically significant difference was found (Table 1). On the other hand, when compared all sex groups in GSH levels statistically significant difference was found (p<0.05), but when compared all sex groups in GPX enzyme activity no statistically significant difference was found (p>0.05) (Table 2).

**Table 1:** The comparison of enzymes activities among age groups in healthy individuals.

| Age | SOD (U/gHb) median(min-max) | CAT (U/gHb )×10⁴ median(min-max) | GPX (U/gHb) median(min-max) | GSH (µmol/gHb) mean±SD | MDA (nmol/ml) median(min-max) |
|---|---|---|---|---|---|
| Group 1 2-11 (n=20) | 6266 (4877-8787) | 17,54 (11,80-24,47) | 50,80 (33,50-89,30) | 0,642±0,090 | 15,82(10,99-23,97) |
| Group 2 12-24 (n=23) | 7644 (5760-9793) | 10,62 (4,83-19,85) | 51,00 (32,70-97,00) | 0,693±0,122 | 25,35 (20,39-37,05) |
| Group 3 25-40 (n=20) | 7164 (4665-11733) | 17,03 (9,03-26,45) | 63,50 (31,50-96,80) | 0,606±0,105 | 25,20 (15,22-31,24) |
| Group 4 41-69 (n=21) | 7299 (5256-9217) | 11,57 (7,29-25,42) | 42,60 (29,40-81,00) | 0,642±0,084 | 25,64 (17,45-38,88) |
| P* | 0,180 | 0,001 | 0,490 | 0,050 | 0,001 |
| P** | - | 0,026[a] | - | 0,038[d] | <0,001[a],<0,001[b],0,025[c] |

P* values were calculated by Kruskal-Wallis or ANOVA test. P** were calculated by post-hoc test. a,b,c,d are the p values for post-hoc test of Groups 1 – 2, Groups 1 – 3, Groups 1 – 4, Groups 2 – 3, respectively. P values were shown when there is a significant difference among groups.

**Table 2:** The comparison of SOD, CAT, GPX, GSH, MDA activities depending on sex within age groups in healthy individuals.

| Group | Age | SOD (U/gHb ) Median (min-max) | CAT (U/gHb)x$10^4$ Median (min-max) | GPX (U/gHb) Median (min-max) | GSH (µmol/gHb) Mean ± SD | MDA (nmol/ml ) Median (min-max) |
|---|---|---|---|---|---|---|
| Group 1 2-11 | Female (n=10) | 6373(4951-8787) | 17,40(11,80-24,30) | 51,35(33,50-83,20) | 0,6336±0,0784 | 15,38(11,80-23,97) |
| | Male (n=10) | 6306(4877-8441) | 17,68(11,80-24,47) | 46,65(35,70-89,30) | 0,6504±0,1035 | 16,03(10,99-23,62) |
| Group 2 12-24 | Female (n=10) | 7721(5948-9793) | 12,99(9,20-18,92) | 60,60(45,30-93,20) | 0,7744±0,0937 | 23,31(20,39-31,40) |
| | Male (n=13) | 7024(5250-8343) | 9,19(4,83-19,85) | 48,70(32,70-97,00) | 0,6316±0,1062 | 27,33(21,16-37,05) |
| Group 3 25-40 | Female (n=10) | 9838(6445-11733) | 20,02(14,22-26,45) | 59,55(31,50-83,00) | 0,6676±0,1299 | 26,09(22,76-31,24) |
| | Male (n=10) | 6497(4665-9217) | 14,69(9,03-23,44) | 67,00(32,50-96,80) | 0,5745±0,0787 | 23,83(15,22-26,52) |
| Group 4 41-69 | Female (n=10) | 7418(5967-8418) | 10,63(7,29-22,76) | 41,50(30,80-73,60) | 0,6623±0,0966 | 24,54(17,45-34,73) |
| | Male (n=11) | 7408(6980-8594) | 13,45(7,79-25,42) | 45,95(29,40-81,00) | 0,6246±0,0712 | 27,29(18,80-38,88) |
| Total sex | Female(n:40) | 7594(4951-11733) | 14,53(7,29-26,45) | 49,50(30,80-93,20) | 0,6807±0,1112 | 23,20(11,80-34,73) |
| | Male (n:44) | 6598(4665-9217) | 13,45(4,83-25,42) | 52,50(29,40-97,00) | 0,6230±0,0932 | 23,65(10,99-38,88) |
| P values | | NS[a,b,d],0,003[c], 0,004[e] | NS[a,d,e], 0,020[b,c] | NS[a,b,c,d,e] | NS[a,c,d], 0,004[b], 0,005[e] | NS[a,d,e], 0,04[b], 0,01[c] |

P values of SOD, CAT, GPX, MDA were calculated by Mann-Whitney test. P values of GSH were calculated by student t test. a, b, c, d, e are the p values for Group 1, Group 2, Group 3, Group 4 and total sex, respectively. NS: Non-Significant. ([a]P:0,847, [b]P:0,367, [d]P:0,360 for SOD; [a]P:0,958, [d]P:0,324, [e]P:0,153 for CAT; [a]P:0,628, [b]P:0,388, [c]P:0,375, [d]P:0,842, [e]P:0,691 for GPX; [a]P:0,659, [c]P:0,118, [d]P:0,321 for GSH; [a]P:0,904, [d]P:0,277, [e]P:0866 for MDA).

## Discussion

Reactive oxygen species are responsible for cell growth, cell differentiation, cell aging and cell death. To date, the relationship between aging and oxidative stress are discussed in scientific terms. Oxidative stress affects biological molecules and is considered to be one of the most important phenomena related to aging. According to authors, oxidative stress theory of aging / received as a marker for aging free radicals and oxidative stress have demonstrated the basic logical consequences of those critical values [15]. While oxygen metabolism is essential to life on the one hand, it has also long-term toxic effects [16]. The authors found a negative correlation in healthy subjects between erythrocyte SOD activity and age. They found increased CAT and GPX activities and MDA levels [17]. Another author investigated CuZn-SOD, GPX, CAT and glutathione reductase (GR) activities in individuals between the ages of 20-89 years [18]. Decreased activity of SOD and GR levels, on the other hand, they did not find age-related of GPX and CAT activities [18]. Another researcher investigated age, gender and smoking effects on SOD, CAT and GPX activities [19]. They observed a decrease in the age-related SOD activity, but they did not find any difference in CAT activity [19]. GPX activity showed an age-related linear increase. SOD and CAT activities in women were higher than men but GPX activity was lower. Antioxidant enzyme activity between smokers and non-smokers showed no difference.

Another researcher reported that aging is a complicated process involving several factors [20]. Two of which are oxidative stress and mitochondrial dysfunction the importance of mitochondrial dynamics in aging is associated with a growing number of age-associated pathogenesis conditions. It is important that we understand the response to oxidative stress and mitochondrial dynamics better so as to develop new therapeutic approaches for the prevention or amelioration of age-associated degenerative diseases.

Studies have revealed that activity of these enzymes can either increase or decrease in the aging process. The studies also reported that there are some regulatory mechanisms in aging tissues to provide an effective antioxidant defence against free radicals, generated at a higher rate during the aging process [18-21]. In our study, the GSH level and GPX activity did not change according to the age, and the difference between the sexes in the GPX activities was not statistically significant (P>0.05). GSH levels were found to be statistically significant. SOD activity was found to be statistically significant in the 25-40 age group as when sexes were compared. CAT activities were found as 12-24 and 25-40 age group statistically significant in the comparison of sexes. MDA levels of groups 12-24, 25-40 and 41-69 compared to group 2-11 were statistically significantly different, but when groups 12-24, 25-40, and 41-69 were compared to each other, no statistically significant differences were found. MDA levels were found as 12-24 and 25-40 age group statistically significant compared with sexes related (Table 2).

The results show that CAT and SOD activities and GSH and MDA levels are affected in aging. Therefore, we recommend that lipid peroxidation may have a role in the pathophysiological alterations of aging.

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