Effect of Age on Antioxidant Enzymes in Women Folk

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ABSTRACT

BACKGROUND: Oxidative stress induces oxidative damage to DNA and other biomolecules which may impair normal functions of tissue cells and lead to human aging and disease especially in women. The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species.

OBJECTIVE: To analyse the age-dependent alteration in the activity of plasma SOD and CAT in the female population of Kashmir.

METHODS: The study group consisted of total 203 normal healthy Kashmiri women. Superoxide dismutase (SOD) and catalase (CAT) protein estimation of each sample was done by Lowry method. Catalase and SOD activity was measured by method of Claiborne (1985) and bishop respectively.

RESULTS: In 20-30 years of age group, catalase activity was 3.37 ± 0.10 ; in 30-40 years of age group 2.92 ± 0.7 , and 2.0 ± 0.07 in > 40 years of age group ($p > 0.05$). In 20-30 yrs of age group; SOD activity was 12.7 ± 0.238 , 19.6 ± 0.028 in 30-40 yrs of age group and 53.7 ± 0.036 in > 40 yrs of age group and this difference in the activities of catalase and SOD was observed to be significant ($p > 0.05$)

CONCLUSION: We conclude that decrease in catalase activity is responsible for increase in oxidative stress with advancing age and increase in SOD enzyme activity with advancement in age of women declines oxidative stress in our population in response to ever increasing oxidative stress in women with advancing age, thus decreasing the overall CAT/SOD ratio with increasing age. JMS 2012;15(2):119-22

Keywords: Catalase, superoxide dismutase, glutathione peroxides, glutathione reductase, reactive oxygen species, fenton reaction.

Oxidative stress is elicited by aerobic metabolism and to cope with this oxidative stress animal and human cells have developed a ubiquitous antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase together with a number of low molecular-weight

antioxidants such as ascorbate, α -tocopherol and glutathione.^{1,2} An increase in ROS-elicited oxidative damage to DNA and other biomolecules may impair normal functions of tissue cells and lead to human aging and disease.^{3,4} Aging is a multifactorial process involving morphological and biochemical changes in single cell and in the whole organism. The exact mechanism responsible for aging is not well understood, but there is enough evidence to suggest a possible relationship between life span and production of free radicals. It has been suggested that aging could be caused by the accumulation of deleterious effects of reactive oxygen species (ROS), throughout the life.⁵ Aerobic

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cells produce ROS as a byproduct of their metabolic processes. The ROS cause oxidative damage to macromolecules under conditions in which the antioxidant defense of the body is overwhelmed.⁶ A certain amount of oxidative damage takes place even under normal conditions; however, the rate of this damage increases during the aging process as the efficiency of antioxidative and repair mechanisms decrease.^{7,8} The correlation between antioxidant capacity and oxidative damage during aging has been reported in several tissues in different species.^{9,10} Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2). Although H_2O_2 is not a radical, it is rapidly converted by fenton reaction into OH radical which is very reactive. Catalase (CAT) metabolizes H_2O_2 into H_2O . Oxidative stress is the primary factor regulating the gene expression of these enzymes.¹¹ However, there are many other factors, such as inflammation, hormonal regulation, and aging that also influence the activity of antioxidant enzymes.¹² In the present study, we report the age-dependent alteration in the activity of plasma SOD and CAT in the Kashmiri population.

Methods

The study was carried out on pre and postmenopausal Kashmiri women of three different age groups. 20-30 yrs, 30-40 yrs and above 40 yrs of age at Department of Clinical biochemistry Sher-i-Kashmir Institute of Medical Sciences (SKIMS) during the period of January 2011 to December 2011. The study group consisted of total 203 normal healthy Kashmiri women, of which 72 women were in the age group of 20-30 yrs, 65 women were in the age group of 30-40 yrs and 66 were of >40 yrs of age. All the participants were non-alcoholic, non-smokers and ambulatory. All persons gave their informed consent for the use of their blood samples for the study. The protocol of study was in conformity with the guidelines of the Institutional Ethical Committee.

Protein estimation

For SOD and Catalase estimation, first protein estimation of each sample was done by Lowry method.¹³ Standard curve was obtained by using bovine serum albumin (50 mg %).

Catalase estimation

Catalase was estimated by using the method of Claiborne (1985).¹⁴ The assay consisted of 100 μ l of serum, 200 μ l 0.019 M of H_2O_2 and 390 μ l of 0.05 M phosphate buffer in a cuvette and then absorbance was read at 240nm using uv spectrophotometer. Then specific activity of each sample was measured in U/min/mg proteins, using the formula:

AA/min/vol of sample/mg of protein/extinction co-efficient x dilution factor

Here; AA = change in absorbance; vol. of sample = 0.1 ml

Extinction coefficient = 9.6×10^3 ; dilution factor = 6.9 ml (volume of assay)

SOD estimation

SOD was estimated by Bichop and Freidrich method.¹⁵ The assay system consisted, 200 μ l of 50 mM sodium carbonate, 400 μ l of 0.1 mM EDTA, 800 μ l of 25 μ M NBT. 800 μ l of 1mM hydroxylamine hydrochloride and 100 μ l of sample in a cuvette. Then incubate for 15 minutes under tungsten lamp. Measure absorbance at 560 nm. Then specific activity of each sample was estimated in U/min/mg of protein, by using the formula:

AA/min/vol of sample/mg of protein/extinction co-efficients x dilution factor

Here; Vol of sample = 0.1 ml; extinction co-efficient = 7.4×10^3 ; dilution factor = 23 ml (volume of assay).

Results

We observed significant variations between the three groups in the activities of enzymes directly involved in ROS scavenging. In particular, in older women (> 40 yrs of age group) the specific activity of catalase, the enzyme that catalyses the removal of hydrogen peroxide, is significantly lower, than young women of 20-30 yrs. The catalase activity decreased as the women advance in age. In 20-30 years of age group, catalase activity was 3.37 ± 0.10 ; in 30-40 years of age group 2.92 ± 0.7 , and 2.0 ± 0.07 in > 40 years of age group (Table 1).

TABLE 1. Antioxidant enzymes in Kashmiri women in three different age groups

Age group	Number	Catalase (U/min/mg of protein)	SOD (U/min/mg of protein)	P-Value
Group 1	72	3.37 ± 0.1	12.7 ± 0.238	0.03
Group 2	65	2.92 ± 0.7	19.6 ± 0.028	
Group 3	66	2.0 ± 0.07	53.7 ± 0.036	

The gradual decline was observed in catalase enzyme activity with increasing age. SOD activity increased as the women advance in age. In 20-30 yrs of age group; SOD activity was 12.7 ± 0.238 , 19.6 ± 0.028 in 30-40 yrs of age group and 53.7 ± 0.036 in > 40 yrs of age group (Table 1).

The changes in SOD and catalase activities cause a reduction in the catalase/SOD ratio. We observed a significant positive correlation between plasma catalase and human age. Also a statistically significant negative correlation was observed between plasma SOD activity and human age ($p = 0.03$).

Discussion

Detrimental effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species. Inactivation and removal of ROS depend on reactions involving the antioxidative defense system. The activity of SOD and catalase ensure an efficient

scavenging action against reactive oxygen intermediates, thus preventing them from rapidly diffusing into oocyte membranes during the period of resumed meiotic activity. It is well known that germ cell membranes are particularly vulnerable to attack by ROS, being very rich in polyunsaturated fatty acid.^{16,17} The capacity of antioxidant defense is determined by the contributions of certain vitamins (A, E, and C), β carotene, reduced glutathione, and antioxidative enzymes. It has been suggested by many authors that oxidative stress is a possible cause for aging. Changes in oxidative damage and antioxidant capacity during aging have been shown in several tissues indifferent species.^{9,10,18} Reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and the hydroxyl radical are known to play a role in organ damage associated with aging and there is higher production of ROS in aged persons than young and middle-aged persons.

In the present study catalase activity decreased as the women advance in age. This is in agreement with a recent study, according to which, low levels of catalase may play a role in the greying process of human hair. Hydrogen peroxide is naturally produced by the body and catalase breaks it down. If there is a dip in catalase levels, hydrogen peroxide cannot be broken down. This causes the hydrogen peroxide to bleach the hair from the inside out.¹⁹

Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzymes that protect cells from ROS. SOD activity was highest in blood of late menopausal group compared with both early and premenopausal group. This result has a positive correlation with a study which also confirms that SOD activity increases with the increasing age of women.²⁰ This increased activity of antioxidant enzymes among aged persons seems to be a compensatory mechanism against high levels of ROS in old age; we hypothesize that this adaptability ensures that oxidation-mediated damage takes place at a rate that determines the pace at which we age, defined by Harman as the 'inborn aging process'.²¹ A recent report of an increase in the activity of erythrocyte membrane plasma membrane redox system also corroborates our present finding of an increase in the activity of plasma SOD.²² Looking at both of the findings (higher erythrocyte PMRS and increased activity of plasma SOD) in conjunction, we hypothesize that the human body has inherent compensatory mechanisms against oxidative stress; however, this capacity is overwhelmed during aging. Our findings also emphasize the need to establish age dependent reference values for SOD and CAT involving their role in different disease conditions. The age-dependent changes in SOD and catalase activities cause a reduction in the catalase/SOD ratio, although in this case we cannot state that the observed decrease in CAT/SOD ratio results in reduced scavenging efficiency, it is important to note that similar age-related ratio decreases found in some mammalian organs strongly affected their response to oxidative stress challenge.^{23,24}

In conclusion, although we are aware of the limited population size of the present study, the major findings of this work is that a different pattern of antioxidant enzymatic defences is seen in women of varied age groups, especially in late menopausal stage in women. This condition might be due to an age-linked altered metabolism. We conclude that increase in SOD enzyme activity with advancement in age of women declines oxidative stress in our population, thus decreasing the overall CAT/SOD ratio with increasing age.

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