

Effect of Aging on Antioxidant Enzyme Status and Lipid Peroxidation

Saxena R*, Lal A.M **

Abstract

Background: Cellular damage by reactive oxygen species including those associated with lipid peroxidation is considered to be a significant factor in the aging process and its consequent sequelae. Although limited information is available about the age related changes in relation with oxidative stress, there is a paucity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes and lipid peroxidation with increase in age.

Aim: The objective of present study was to estimate the levels of antioxidant enzymes and malondialdehyde in the subjects of different age groups and to determine the variation in their levels with increasing age.

Methods: In the present study, 150 healthy individuals were selected and antioxidant enzyme status (superoxide dismutase, catalase, glutathione peroxidase and ceruloplasmin) and malondialdehyde levels were measured by using standard methods. Out of 150 healthy subjects, 100 individuals were categorized into two groups: Group I (40-55 years) and Group II (\geq 56 years) and statistically compared with that of 50 controls (20-30 years) by using student's t-test.

Result: Erythrocyte superoxide dismutase, catalase and glutathione peroxidase activity were significantly low ($p < 0.001$) in Group I and Group II as compared to healthy controls whereas ceruloplasmin level was increased insignificantly ($p < 0.01$) and malondialdehyde level was increased significantly ($p < 0.05$) in both the study groups as compared to healthy controls.

Conclusion: Our findings indicate that alteration in antioxidant enzyme status and increased production of MDA are excellent marker of oxidative stress during aging. Thus, the diet rich in antioxidant or antioxidant supplementation could be beneficial in delaying the aging process.

(Journal of The Indian Academy of Geriatrics, 2006; 2:53-56)

Introduction

Aging is an universal and inevitable, normal biological phenomenon resulting from tissue damage by free radicals leading to progressive morphological and physiological deterioration of the organs that are often accompanied with frequent attacks of various degenerative diseases.¹ Many physiological processes are known to result in the production of oxygen free radical e.g. enzymatic action (NADPH oxidase, xanthine oxidase system), electron transport processes within the mitochondria, arachidonic acid metabolism and the activation of phagocytic cells. Antioxidant defense

system of body reduces or eliminates the free radicals e.g. superoxide ($O_2^{\cdot-}$), hydroxyl (OH^{\cdot}), hydrogen peroxide (H_2O_2) and peroxy free radical (ROO^{\cdot}). In early age, the generation of free radicals appear to be approximately in balance with the antioxidant defense system but as the age progresses this balance is upset because of reduction in antioxidant reserve and excessive production of free radicals which play a crucial role in age related changes and its consequent sequelae.² The antioxidant defense system which protects the biomolecules against potentially damaging effects of free radicals include antioxidants and antioxidant enzymes e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), ceruloplasmin, uric acid, vitamin C and vitamin E etc. Superoxide dismutase (SOD, EC: 1.15.1.1) catalyses the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen. Hydrogen peroxide is further detoxified by either heme containing enzyme catalase (CAT, EC: 1.11.1.6) or selenium containing enzyme glutathione peroxidase (GSHPx, EC: 1.11.1.9)³. Ceruloplasmin (E.C:1.16.3.1), is a blue

*Department of Biochemistry, M L N Medical College, Allahabad
 Department of Biochemistry, AAI-DU, Allahabad.

Address for Correspondence

Dr Rahul Saxena
 C/o Dr Geeta Jaiswal
 R/o 234, Attarsua, Allahabad.
 E-mail : Raj-Kusum22@rediffmail.com

colored copper binding protein, function as antioxidant enzyme by virtue of its ferroxidase activity and scavenges superoxide anion radical.⁴

Lipid peroxidation (LPO) is a chain reaction, initiated with attack of free radical on phospholipid or polyunsaturated fatty acid of membrane of cellular or subcellular organelle resulting in the generation of complex mixture of aldehydes, ketones and polymerization products which react and destroy the biomolecules, enzymes and nucleic acid leading to aging process. Malondialdehyde (MDA) is the most abundant and excellent marker of lipid peroxidation among reactive aldehydes.⁵ Although limited information is available about the age related changes in relation with oxidative stress, there is a paucity of satisfactory explanation regarding the alteration in the level of antioxidant enzymes (SOD, CAT, GSHPx and ceruloplasmin) and lipid peroxidation with increase in age. Therefore, the overall objective of present study was to estimate the level of antioxidant enzymes and malondialdehyde in the subjects of different age groups and to determine the variation in their levels with increasing age.

Material and Methods

In the present study, 150 healthy, non supplemented volunteers of both sex and different age groups were taken. These subjects were categorized into three groups depending upon age i.e. control group (younger people) included 50 healthy subjects of age group 20 – 30 years, Group I included 50 healthy subjects (middle aged people) of age group 40 – 55 years and Group II included 50 healthy subjects (elderly aged people) of age group 56 years onwards. Venous blood was collected in EDTA vial from 7–10 a.m. (fasting sample) from each subject after collecting the information of age, sex, height, weight, blood pressure and confirmation of healthy state. Height and weight were measured with subject barefoot and light dressed. The

body mass index (BMI) was calculated as $BMI = \text{weight (Kg)} / \text{Height (metre}^2\text{)}$. Individual with BMI > 25, blood pressure >120/80 mmHg and smokers were excluded from the study. Samples were processed immediately. After preparation of hemolysate erythrocyte SOD, Catalase and GSHPx activity were measured by Marklund and Marklund's method, Goth's method and Beutler's method respectively.^{6,7,8} Serum ceruloplasmin level and MDA level were measured by Ravin's method and Uitley's method spectrophotometrically.^{9,10} The data from both the study group subjects and controls were compared using student's t-test and values were expressed as Mean ± Standard Deviation (SD).

Result

The anthropometric measurements and the levels of antioxidant enzymes and malondialdehyde in the subjects of various age groups are depicted in Table 1 and 2 respectively. All the study group subjects were normal, non-obese and normotensive. The erythrocyte SOD activity in controls (younger people), group I and group II were 1932.24 ± 342.46 , 1338.46 ± 231.53 and 1226.97 ± 265.32 U/gm Hb respectively. Catalase activity were 52.7 ± 9.5 , 35.4 ± 6.8 and 32.9 ± 8.4 KU/L and Glutathione peroxidase activity were 36.4 ± 8.6 , 27.71 ± 4.3 and 25.72 ± 6.2 IU/gm Hb respectively in controls, group I and group II subjects. On comparing these levels, it has been observed that these levels were significantly low in group I and group II (Table 2, $p < 0.001$, $p < 0.05$) as compared to healthy controls but these values do not differ significantly in group I as compared to group II. Serum Ceruloplasmin level was increased insignificantly in both the study groups ($P < 0.01$) as compared to healthy controls (5.21% and 12.84%) whereas MDA levels were found to be significantly high ($p < 0.05$) in both the study groups as compared to healthy controls (23.21% and 29.42%).

Discussion

Cellular damage by reactive oxygen species

Table 1: Age and Anthropometry of various age groups i.e. younger, middle aged and elderly subjects (Mean±SD).

S.No.	Particulars	Control group (n=50)	Group I (n=50)	Group II (n=50)
1	Age (years)	25±5.0	47.5±6.5	63.0±8.0
2	M:F ratio	1:1	1:1	1:1
3	Height (meter)	1.62± 0.08	1.59± 0.07	1.60±0.05
4	Weight (Kg)	56.8 ±9.83	59.2 ±4.8	61.4± 3.2
5	B.M.I. (Kg/m ²)	21.30± 2.8	23.42± 3.6	23.96± 2.4
6	Systolic blood pressure (mmHg)	104 ±12.0	112 ±6.2	118 ±8.4
7	Diastolic blood pressure (mmHg)	76.0±2.8	74.0± 5.6	70.0±4.2

Table 2: Antioxidant enzyme status and malonaldehyde level of various age group subjects (Mean±SD).

S.No.	Particulars	Control group (n=50)	Group I (n=50)	Group II (n=50)
1	Age (years)	20-30	40-55	≥ 56.0
2	SOD (U/gm Hb)i) Mean±SDii) % Decrease	1932.24 ± 342.46 -	1338.46 ± 231.53* 30.73	1226.97±265.32 * 36.50
3	Catalase (KU/L) i) Mean±SDii) % Decrease	52.7 ± 9.5 -	35.4± 6.8* 32.74	32.9± 8.4* 37.55
4	GSHPx (IU/gmHb) i) Mean±SDii) % Decrease	36.4 ±8.6 -	27.71± 4.3 ^{3/4%} 23.87	25.72± 6.2 ^{3/4%} 28.34
5	Ceruloplasmin (mg%) i) Mean±SD ii) % Increase	21.87± 4.3 -	23.01± 3.8** 5.21	24.68± 5.6** 12.84
6	Malonaldehyde (μ mol MDA/ml) i) Mean±SD ii) % Increase	1.12± 0.07 -	1.38± 0.09 ^{3/4%} 23.21	1.44 ±0.10 ^{3/4%} 29.42

*P<0.001 : Highly significant; ^{3/4%} P<0.05 : Significant; **P<0.01 : Insignificant

including those associated with lipid peroxidation is generally believed to be a significant factor in the aging process and its consequent sequelae. Antioxidants and antioxidant enzymes, present in the body, destroy these free radicals. The primary intracellular antioxidant enzyme responsible to scavenge free radicals includes SOD, catalase, GSHPx and ceruloplasmin.³ Alteration in their activities directly influences the biological properties of body. In the present study, erythrocyte SOD activity was significantly low in middle aged and elderly people (30.73% and 36.50% low) as compared to younger people which direct towards reduction in its protective and O₂ radical scavenging action in middle aged and elderly people. The diminished activity of SOD among these subjects could be explained on the basis of its progressive enzyme inactivation by resultant product of dismutation reaction i.e. H₂O₂ or due to increase in the glycosylation of SOD with aging. Our findings were in concordance with the findings of Ceballos-Picot et.al. who demonstrated negative correlation between age and SOD levels in human erythrocytes.¹¹ Bianchi et.al. also observed a linear quadratic effect of age on SOD activity in whole blood

and concluded that the activity of this enzyme in human blood decreases with age.¹²

H₂O₂ produced via dismutation reaction of O₂ is mainly removed by catalase and glutathione peroxidase. Both the enzyme activities were also found to be decreased in group I and group II (i.e. p< 0.001 and p< 0.05, Table 2) as compared to controls which could be explained either by their decreased synthesis or rapid consumption in protecting the cells from H₂O₂ mediated damage with increasing age. Our findings were quite similar to the findings of Ji et.al. and Mote et.al. who have also reported age related decrease in catalase and GSHPx activity.^{13, 14} Reduction in antioxidant enzyme activities are generally associated with increased risk of age related complications such as cardiovascular disease, hypertension and neurological disorders.^{15, 16, 17}

Superoxide anion (O₂⁻) scavenging action of SOD can be mimicked by other copper containing enzyme ceruloplasmin, which has also the capacity to scavenge O₂ radical. In the present study, serum ceruloplasmin level was found to be increased insignificantly in both

the study groups as compared to healthy controls, which indicate that ceruloplasmin protects the tissues against the deleterious effects of oxygen free radical and compensates for the loss of SOD activity, which occurs due to oxidative stress in elderly subjects. Although age related changes in ceruloplasmin level have been the subject of intensive investigation, its ambiguous property as an antioxidant enzyme as well as acute phase protein are well reported in previous studies.^{18,19} However, it is still unclear whether the altered activity of antioxidant enzymes is the cause or the consequence of increased oxidative stress during aging.

Reduced enzyme activity therefore means increased production of H_2O_2 or incomplete scavenging of O_2^- leading to further destruction i.e. lipid peroxidation via formation of highly reactive OH radical as a consequence of Haber- Weiss reaction with H_2O_2 . Malondialdehyde (MDA), the most abundant reactive aldehyde derived from lipid peroxidation has been implicated as the causative agents in cytotoxic processes related to aging and its associated risk of cardiovascular disease most probably by inducing oxidative modification in cell membrane and low density lipoprotein molecules.¹⁶ MDA level was also found to be significantly high in both the study groups ($p < 0.05$, Table 2) as compared to control which indicate that aging is closely associated with lipid peroxidation mediated destruction in cell, subcellular organelles and biomolecules. Our findings were also in agreement with the findings of Goldberg.²⁰

The antioxidant enzymes which are excellent markers of oxidative stress change with aging. Increased oxidative stress contributes to various features of aging. Though changes occurring during aging cannot be avoided but may be delayed and controlled to some extent by exogenous antioxidant supplementation. It may prevent or postpone the onset of age related degenerative diseases. The present evidence is strong enough to have convinced nutritionists that daily consumption of fruits and vegetables rich in antioxidants should be increased with age in order to sustain the harmful action of free radicals during aging.

References

- 1 Singh S, Saxena R, Lal AM. Influence of aging on plasma ascorbate level. *Natl Acad Sci Lett* 2005; 28: 125-127.
- 2 Sohal RS, Allen RG. Oxidative stress as a causal factor in differentiation and aging, a unifying hypothesis. *Exp Gerontol* 1990; 25: 499-522.
- 3 Sen CK. Oxygen toxicity and antioxidants: state of the art. *Indian J Physiol Pharmacol* 1995; 39: 177 – 196.
- 4 Winyard PG, Hider RC, Brailsford S, et al. Effects of oxidative stress on some physicochemical properties of ceruloplasmin. *Biochem J* 1989; 258: 435-445.
- 5 Das D. Vitamins and coenzymes. Biochemistry, 11th edn., Academic Publishers, Kolkata. 2002, p. 243 – 288.
- 6 Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974; 47: 469 – 474.
- 7 Goth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chem Acta* 1991; 196: 143 - 151.
- 8 Beutler E. Red cell metabolism. A Manual of Biochemical Methods. New York. Grune and Stratton Inc. 1971, 3rd Edn, p 112-114.
- 9 Ravin HA. An improved colorimetric enzymatic assay of ceruloplasmin. *J Lab Clin Med* 1961; 58: 161-168.
- 10 Utley HG, Bernhein F, Hochstein P. Effect of sulphhydryl reagents on peroxidation in microsomes. *Arch Biochem Biophys* 1967; 118 : 28.
- 11 Ceballos-Picot L, Trivir JM, Nicole A, et al. Age correlated modification of copper-zinc superoxide dismutase and Glutathione related enzyme activities in human erythrocytes. *Clin Chem* 1992; 38: 66-70.
- 12 Bianchi MS, Bianchi NO, Blazon AD. Superoxide dismutase activity and superoxide dismutase-1 gene methylation in normal and tumoral human breast tissues. *Cancer Genet Cytogenet* 1992; 59: 26-29.
- 13 Ji LL, Dillon D, Wu E. Myocardial aging antioxidant enzyme system and related biochemical properties. *Am J Physiol* 1991; 261: 386-392.
- 14 Mote PL, Grizzle JM, Walford RL, et al. Age related down regulation of hepatic cytochrome P -450, P -450, catalase and Cu Zn superoxide dismutase RNA. *Mech Ageing Dev* 1990; 53: 101-110.
- 15 Kharb S. Low blood glutathione levels in acute myocardial infarction. *Indian J Med Sci* 2003; 57:335-337.
- 16 Tandon R, Sinha MK, Garg H, et al. Oxidative stress in patients with essential hypertension. *Natl Med J India* 2005; 18:297-299.
- 17 Sudha K, Rao AV, Rao S, et al. Lipid peroxidation, hemolysis and antioxidant enzymes of erythrocytes in Stroke. *Indian J Physiol Pharmacol* 2004; 48:199–205.
- 18 Verma VK, Ramesh V ,Tiwari S. Role of bilirubin, vitamin C and ceruloplasmin as antioxidants in coronary artery disease. *Ind J Clin Biochem* 2005; 20: 68 – 74.
- 19 Klipstein-Grobusch K, Grobee DE, Koster JF. Serum ceruloplasmin as a coronary risk factor in the elderly. The Rotterdam study. *Br J Nutr* 1999; 81: 139 – 144.
- 20 Goldberg ED, Bowen VT, Farrington JW, et al. The mussel watch. *Environ Conservation* 1978; 5: 101-125.