International Journal of Research in Medical Sciences
Fasna KA et al. Int J Res Med Sci. 2017 Nov;5(11):4826-4831
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20174928

Original Research Article

Oxidative stress in ageing

Fasna K. A.[1]*, Geetha N.[2], Jean Maliekkal[3]

[1]Department of Physiology, KMCT Medical College, Manassery, Mukkam, Calicut, Kerala, India
[2]Department of Physiology, Government TD Medical College, Alappuzha, Kerala, India
[3]Department of Physiology, Government Medical College, Manjeri, Kerala, India

Received: 21 August 2017
Accepted: 20 September 2017

***Correspondence:**
Dr. Fasna K. A.,
E-mail: drfasna@gmail.com

ABSTRACT

Background: Ageing is characterized by a gradual decline in body functions and decreased ability to maintain homeostasis. The free radical theory of ageing proposed by Harman D states that ageing is a result of cumulative damage incurred by free radical reactions. Free radicals are highly reactive molecular species with unpaired electrons; generated in the body by several physiological processes. Prime target to free radical attack are the polyunsaturated fatty acids of cell membranes causing lipid peroxidation. The free radicals are neutralized by the exogenous and endogenous antioxidant systems. Oxidative stress occurs when large number of free radicals are produced or the antioxidant activity is impaired. The present study is focused to find out the role of oxidative stress in ageing.
Methods: A cross sectional observational study was undertaken to assess the oxidative stress in ageing; by determining the levels of lipid peroxidation product- malondialdehyde (MDA), the antioxidants- superoxide dismutase (SOD) and ceruloplasmin in various age groups. 150 healthy subjects were selected randomly and categorised into three different age groups of 20-30 years, 40-59 years and 60-90 years; with 50 subjects in each group. Results were expressed as mean ± standard deviation.
Results: a significant elevation in serum MDA level and a decline in SOD were observed in 40-59 years and 60-90 years age groups. However, an elevated ceruloplasmin level was found in the above age groups.
Conclusions: Aforementioned observations are suggestive of an association between oxidative stress and the progression of ageing process.

Keywords: Ageing, Antioxidants, Ceruloplasmin, Oxidative stress, MDA, SOD

INTRODUCTION

Ageing is a universal biological phenomenon. Seen as a time related deterioration of the physiological functions leading to a decrease in the ability of the cell to withstand stress.[1] Geriatric population in general includes the persons who are 60 years and above. India has the second largest population of older adults in the world next to China. Around 70 million people were in this age group in 2000; and by 2020 it will be 177million.[2] With this picture; problems of ageing have now become an important social issue and of great public health concern. Ageing affects organs and tissues of a given organism in different ways, resulting in different rates of functional decline. Common health problems encountered in old age include increased susceptibility to infection, inability to cope with physical and psychological stress, various types of cognitive disorders, degenerative arthritis, atherosclerotic and vascular diseases of heart and brain, malignancies affecting various organs etc.[3,4]

The causative factors for ageing are yet to be defined and no single adequate molecular explanation for ageing is currently available. It is proposed that the mechanisms are likely to be multifactorial, environmentally influenced and species specific. Various theories have been advanced about the possible causes of ageing. The free radical theory of ageing proposed by Harman D has received widespread attention. It states that ageing is a result of cumulative damage incurred by free radical reactions.[5]

Free radicals or reactive oxygen species (ROS) are highly reactive molecular species with unpaired electrons. They are generated in the body by several physiological processes like enzymatic reactions, electron transport processes within the mitochondria, arachidonic acid metabolism and the activation of phagocytic cells. Most important ROS generated are superoxide anion, hydroxyl radical and hydrogen peroxide. Normally exogenous and endogenous antioxidant systems perform the function of neutralizing of these ROS thus limiting their activity. There are enzymatic (like superoxide dismutase (SOD), catalase, glutathione peroxidase and ceruloplasmin) and non-enzymatic (like vitamin E, C, albumin, uric acid, glutathione) antioxidants.[6]

Normally a delicate balance exists between the pro-oxidants that generate ROS and the antioxidant defense system which removes these ROS. In the younger age group, the generation of free radicals appears to be in balance with the antioxidant defense system. However, as the age progresses this balance is upset due to the reduction in the activity of antioxidant reserve and the increased production of free radicals. Oxidative stress occurs when large amount of ROS is produced or the antioxidant activity is impaired.[6,7] Abdoljalal Marjani et al reported enhanced oxidative stress with ageing.[8]

Prime target to free radical attack are the polyunsaturated fatty acids (PUFA) which are the major constituents of cellular and subcellular membranes causing lipid peroxidation. Lipid peroxidation leads to the formation of several metabolites of aldehydes, ketones and several polymerization products. MDA is one of the most frequently used biomarkers that provide an indicator of the overall lipid peroxidation level and oxidative stress7. Gil P et al and Akila V et al have reported a significant increase in MDA levels with increase in age.[9,10]

Among the enzymatic antioxidants SOD is the principal chain breaking antioxidant enzyme. It scavenges superoxide anions by catalyzing the conversion of superoxide radicals to hydrogen peroxide which is then converted to water by catalase and glutathione peroxidase. Guemouri et al reported a decrease in SOD levels in elderly in their study.[11] Ceruloplasmin is an important extracellular enzymatic antioxidant. It acts as an antioxidant through its ferroxidase activity and it scavenges superoxide anion radical. Some investigators have reported an elevation in serum ceruloplasmin levels with ageing.[6]

Recently the role of oxidative stress in the development of age related disorders have been documented by several investigators. The degree of oxidative stress in elderly is intensified when they have associated diseases such as essential arterial hypertension, diabetes mellitus, Alzheimer's disease etc. Rahul Saxena and Geeta Jaiswal reported the beneficial role of exogenous antioxidant supplementation in reducing the lipid peroxidation associated with ageing process.[10,12,13] The aim of this study was to find out the role of oxidative stress in ageing.

METHODS

A cross sectional observational study was undertaken to assess the oxidative stress in ageing; by determining the alterations in the levels of lipid peroxidation product-malondialdehyde (MDA), antioxidants - superoxide dismutase (SOD) and ceruloplasmin in various age groups. Approval from the institutional ethics committee was obtained. The study was conducted for a period of 9 months from March 2010 to November 2010 at government medical college, Kozhikode.

Selection of subjects

In the present study 150 healthy subjects were selected from among the hospital staff, students and bystanders of patients admitted in the wards at government Medical College, Kozhikode. The subjects selected were not on any medication or supplemental therapy.

After getting informed consent, a detailed history was taken and screening was done using a proforma. Both males and females were included in equal proportion. Subjects were selected randomly and categorized into three groups with 50 subjects in each group:

- Group 1: included young controls of 20-30 yrs of age,
- Group 2: included middle aged subjects of 40-59 yrs age,
- Group 3: included elderly subjects of 60-90 yrs of age.

Subjects with history of hypertension, diabetes mellitus, renal disease, heart disease, stroke, neurodegenerative diseases, diarrhoea or fever (during the previous 1 week) were excluded from the study. Body mass index was calculated and those with BMI > 25 were excluded from the study.

Disposable syringes and needles were used to collect blood samples from subjects. Under all aseptic precautions 7 ml of blood was drawn from the antecubital vein and was transferred into clean dry bottles. Blood was allowed to clot and the serum separated by centrifugation at 3000 rpm for 15 minutes.

Serum MDA and SOD were assayed by using UV-Vis spectrophotometer 118 (systronics). Serum ceruloplasmin level was analyzed using photoelectric colorimeter (systronics 114).

Statistical analysis has been done to determine the differences between the groups. The results are summarized in Tables 1-3.

Data was analyzed using statistical package for social sciences (SPSS) version 16. Results were expressed as mean ± standard deviation. Mean differences between the groups were analyzed using ANOVA (analysis of variance). It is used to test whether there is significant difference among two or more independent groups. The p value of < 0.05 was taken as the level of significance.

RESULTS

The product of lipid peroxidation- serum MDA was significantly high in middle aged people (84.58 ± 8.62) and elderly (138.57 ± 20.25); when compared to that of young controls (80.28 ± 12.95) Table 1.

Table 1: Comparison of serum MDA levels in normal subjects of three age groups.

	MDA (nmol / dl)			
	Group 1 young controls (20-30 yrs)	**Group 2 middle aged (40 - 59 yrs)**	**Group 3 elderly (60- 90 yrs)**	**P-Value**
Mean ± SD	80.28 ± 12.95	84.58 ± 8.62	-	0.442 (Not significant)
	80.28 ± 12.95	-	138.57±20.25	0.001 (Significant)
	-	84.58 ±8.62	138.57±20.25	0.001 (Significant)

n=150

Table 2: Comparison of serum SOD levels in normal subjects of three age groups.

	SOD [u/ ml]			
	Group 1 (20-30 yrs)	**Group 2 (40 – 59 yrs)**	**Group 3 (60- 90 yrs)**	**P value**
Mean± SD	4.21 ± 1.49	2.9 ± 1.4	-	0.001 (Significant)
	4.21 ± 1.49	-	1.01 ± 0.5	0.001 (Significant)
	-	2.9 ± 1.4	1.01 ± 0.5	0.001 (Significant)

Table 3: Comparison of serum ceruloplasmin levels in normal subjects of three age groups.

	Ceruloplasmin (mg %)			
	Group 1 (20-30 yrs)	**Group 2 (40–59yrs)**	**Group 3 (60- 90 yrs)**	**P value**
Mean ± SD	29.52±4.42	33.16±4.75	_	0.003(significant)
	29.52±4.42	_	45.19±6.61	0.001(significant)
	_	33.16±4.75	45.19± 6.61	0.001(significant)

A significant decrease in the antioxidant enzyme SOD was observed in middle aged people (2.9 ±1.4) and elderly (1.01 ± 0.5) than that of young controls (4.21± 1.49) Table 2.

The ceruloplasmin level in middle aged (33.16 ± 4.75) and elderly (45.19 ± 6.61) were found to be high when compared to that of young controls (29.52 ±4.42). The inter group differences were statistically significant Table 3.

DISCUSSION

The twenty-first century is often called the 'era of ageing'. Since 1950, the proportion of the world's population aged 60 and above has changed from one in thirteen to one in ten, with reports stating that some of the developing countries are ageing faster than the developed countries. A demographic transition is now seen with a shift from high mortality/high fertility to low mortality/low fertility pattern resulting in an increased proportion of older people in the total population with a consistently increased old age dependency ratio. India is also showing such a demographic transition.[14] These changes in population have significant social, economic and political effects on the society.

Ageing is a complex process characterized by a gradual decline in body functions and decreased ability to maintain homeostasis. It affects all physiological processes. Decline in the function of most organs begin to occur by the third and fourth decades of life, and this deterioration progresses with age. Ageing has been shown to be associated with increased free radical (ROS) activity. Oxidative stress ensues when large number of free radicals produced in the cells overwhelm the antioxidant protective mechanisms of cells and tissues. Increased oxidative stress and its

definitive role in the age-related diseases is now a well-documented fact.[15] The present study was conducted to determine the alterations in oxidant– antioxidant status with ageing.

The mean MDA levels in young controls (group 1), middle aged (group 2) and elderly (group 3) subjects were 80.28 ± 12.95, 84.58 ± 8.62 and 138.57 ± 20.25 nmol/dL respectively (table 1, figure 1). A progressive increase in serum MDA levels was seen with increase in age. Significantly higher values were obtained in elderly when compared to that of young controls and middle-aged groups, indicating the presence of increased oxidative damage with ageing. Similar findings were also reported by Kuldip singh et al, Kasapoglu et al and M A dak and S Nazri. Serum MDA is an excellent marker of lipid peroxidation and oxidative stress.[14,16,17]

ROS/free radicals produced in the body as a byproduct of aerobic metabolism damage cellular macromolecules that include lipids, proteins, DNA and mitochondrial components. The accumulation of these oxidative damages may be a major contributory factor in cellular ageing. Lipid peroxidation is an important biological consequence of oxidative cellular damage. It is a chain reaction and leads to generation of more and more free radicals which in turn cause further peroxidation of other PUFA and results in greater cell damage and dysfunction.[7]

Increased oxidative damage with ageing as reflected by elevated MDA levels could result from repeated exposure to ionizing radiations, mitochondrial dysfunction and reduction in antioxidant defense mechanisms. Mitochondria are the major source of ROS/ free radicals in the body. Age related accumulation of mitochondrial DNA mutations and protein oxidation products in mitochondria can lead to insertion of altered enzyme components into the electron transport chain. This in turn results in an increased rate of free radical production that sets off a "vicious cycle" of exponentially increasing oxidative damage and dysfunction.[18]

Cell membranes which are made up of large amounts of PUFA are highly susceptible to oxidative attack. Saxena etal1 reported that lipid peroxidation contributes to local membrane destabilization that alters the proper trafficking of intracellular vesicles, phagocytosis, degranulation, antigen presentation, receptor mediated ligand uptake etc. leading to age related deterioration in many cellular functions. Lipid peroxidation has been implicated in the pathogenesis of numerous age-related diseases such as diabetes mellitus, hypertension, CAD, senile cataract, parkinsonism and alzheimer's disease.[10,19-22]

Depletion of antioxidants is an indirect marker of oxidative stress during ageing. The activity of SOD, a superoxide radical scavenging enzyme was found to be significantly decreased in elderly (1.01 ± 0.5 U/mL) than that of young controls (4.21±1.49 U/mL) and middle-aged subjects (2.9 ± 1.4 U/mL). The decrease in SOD in middle aged subjects was also significant when compared to that of young controls (Table 2). Gil P et al and B Ozbay et al also observed a decrease in SOD activity with ageing.[19,23]

Superoxide dismutase is the first line of defense against the superoxide radical, which is formed from molecular oxygen by single electron transfer. This enzyme converts the highly reactive superoxide radicals (O^{2-}) into less toxic hydrogen peroxide and decreases cell damage. Hydrogen peroxide is further detoxified either by catalase or by glutathione peroxidase to water and molecular O_2. The decreased levels of SOD activity with ageing reflect the cellular damage due to accumulation of O_2^-. The diminished activity of SOD may be due to its progressive inactivation by H_2O_2, or increased glycosylation of SOD with ageing.[6]

Free iron and copper are powerful catalysts of free radical generating reactions. By binding copper, ceruloplasmin prevents free copper from catalyzing reactions that cause oxidative damage. The ferroxidase activity of ceruloplasmin facilitates iron loading into transferrin and prevents free ferrous ions from participating in reactions that generate toxic OH- and H_2O_2 radicals (fenton reaction).[24] The mean ceruloplasmin levels in elderly (45.19 ± 6.61 mg%) and middle-aged group (33.16 ± 4.75 mg%) were significantly higher than that of young controls (29.52 ± 4.42 mg%) - (table 3, figure 3). Our findings were in agreement with those obtained by Daimon et al and king et al.[25,26]

It has been known that the antioxidants are under homeostatic control. A decrease in a particular antioxidant can be compensated by an increase in a different one. Ceruloplasmin has the capacity to scavenge superoxide radicals which mimics the superoxide scavenging actions of SOD. The elevation in Ceruloplasmin level occurs as a compensatory response to increased oxidative stress during ageing.[27] Aforementioned observations are suggesting that there might be an association between oxidative stress and the progression of ageing process. This may induce biomolecular deterioration and produce disturbances in the levels of sodium, potassium, calcium and magnesium which are essential for maintaining fluid-electrolyte balance, pH, and proper functioning of various enzymes in our body.

Studies have shown that the most effective way of prolonging life span is caloric restriction. This may decrease the production of mitochondrial O^{2-} and H^2O^2, and increase the production of antioxidant defenses. Also, it has been proved that supplementation of vitamins with antioxidant activity can improve the functioning of endogenous antioxidant system. An increase in the SOD activity and a decrease in MDA levels after antioxidant - vitamin E supplementation in both middle aged and elderly have been reported in a recent study.[24] For the primary prevention of the diseases of middle to old age, it is essential to adopt a healthy lifestyle from an early age as in childhood or in the young adult (Report of a Joint

WHO/FAO Expert Consultation). A healthy lifestyle includes regular physical exercise, nonsmoking, and a nutrient-rich low-calorie diet to maintain a moderately lean body weight. The composition of the diet is also important, since there is good evidence that a vegetarian diet (rich in antioxidants), the Mediterranean diet (high in olive oil with monounsaturated fatty acids), and the Okinawan diet (high in fruits and vegetables plus omega-3 fatty acids in fish) are beneficial by delaying age-associated diseases.[28]

ACKNOWLEDGEMENTS

We would like to thank all who have supported us for the successful completion of this work. In particular we would like to express our sincere gratitude to all the subjects who cooperated with us, teaching and nonteaching staff members.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Saxena R, Lal AM. Culprit effect of altered total antioxidant status and lipid peroxidation mediated electrolyte imbalance on aging. J Ind Acad Geriat. 2007;3:137-144.
2. Moharana PR, Sahani NC, Sahu T. Health status of geriatric population attending the preventive geriatrics clinic of a tertiary health facility. J Community Medic. 2008;4(2):41-5.
3. Dey AB. Emerging Challenges of Old Age Care in India. J Ind Acad of Geriat. 2006;2(4):134-5.
4. Singh K, Kaur S, Kumari K, Singh G, Kaur A. Alterations in lipid peroxidation and certain antioxidant enzymes in different age groups under physiological conditions. J Hum Ecol. 2009;27(2):143-7.
5. Kasper, Braun wald, Fauci, Hauser Longo, Jameson. Harrison's Principles of internal medicine, McGraw Hill. 1(16);43-46.
6. Saxena R, Lal AM. Effect of Aging on antioxidant enzyme status and lipid peroxidation. J Ind Acad Geriartics. 2006;2(2):53-6.
7. Vasudevan DM, Sreekumari S, Vaidyanathan K. Textbook of biochemistry for medical students. JP Medical Ltd. 2013; 4:338-342.
8. Marjani A. Age-related alterations of plasma lipid peroxidation and erythrocyte superoxide dismutase activity in different age groups of Gorgan City, Iran. Saudi medical J. 2005;26(10):1647-8.
9. Gil P, Farinas F, Casado A, Lopez-Fernandez E. Malondialdehyde: a possible marker of ageing. Gerontology, 2002 Jul; 48(4): 209-14.
10. Akila V, Prashant H, Harishchandra, Vivian D'souza, Benedicta D'souza. Age related changes in lipid peroxidation and antioxidants in elderly people. Ind J clinic Biochem. 2007; 22(1): 131-134.
11. Guemouri LY, Artur Y, Herbeth B, Jeandel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clinical Chemistry. 1991;37(11):1932-7.
12. Suresh DR, Kumaran S, Annam V. Age related changes in Malondialdehyde: Total Antioxidant Capacity Ratio–a novel marker of oxidative stress. Int J Pharma BioSciences. 2010;1:1-6.
13. Saxena R, Jaiswal G. Vitamin E, markers of oxidative stress and nitric oxide levels in senescence. J Indian Acad Geriat. 2010;6:71.
14. Ravishankar AK. Ageing and family support of elderly in South India. Ind J Gerontol. 2010;24(4):482-500.
15. Pandey KB, Rizvi SI. Markers of oxidative stress in erythrocytes and plasma during aging in humans. Oxidative medicine and cellular longevity. 2010;3(1):2-12.
16. Kasapoglu M, Özben T. Alterations of antioxidant enzymes and oxidative stress markers in aging. Experimental gerontology. 2001;36(2):209-20.
17. Adak M, Nazri S. Age related study of serum lipid peroxidation and antioxidant status. J Institute Medic. 2007;29(1).
18. Shigenaga MK, Hagen TM, Ames BN. Oxidative damage and mitochondrial decay in aging. Proceedings of the National Academy of Sciences. 1994;91(23):10771-8.
19. Kedziora-Kornatowska K, Czuczejko J, Pawluk H, Kornatowski TO, Motyl JA, Szadujkis-Szadurski LE, Szewczyk-Golec KA, Kedziora J. The markers of oxidative stress and activity of the antioxidant system in the blood of elderly patients with essential arterial hypertension. Cell Mol Biol Lett. 2004;9(4A):635-41.
20. Schmuck A, Fuller CJ, Devaraj S, Jialal I. Effect of aging on susceptibility of low-density lipoproteins to oxidation. Clinical chemistry. 1995;41(11):1628-32.
21. Chakraborty I, Kunti S, Bandyopadhyay M, Dasgupta A, Chattopadhyay GD, Chakraborty S. Evaluation of serum zinc level and plasma SOD activity in senile cataract patients under oxidative stress. Ind J Clinic Biochem. 2007;22(2):109-13.
22. Hwang IK, Yoon YS, Yoo KY, Li H, Choi JH, Kim DW, Yi SS, Seong JK, Lee IS, Won MH. Differences in lipid peroxidation and Cu, Zn-superoxide dismutase in the hippocampal CA1 region between adult and aged dogs. J Veterinary Medic Sci. 2008;70(3):273-7.
23. Özbay B, Dülger H. Lipid peroxidation and antioxidant enzymes in Turkish population: relation to age, gender, exercise, and smoking.Tohoku J Exp Medic. 2002;197(2):119-24.
24. Saxena R, Lal AM. Influence of vitamin E supplementation on ageing. J Ind Academy of Geria. 2008; 4(2): 55-59.
25. Daimon M, Susa S, Hasegawa K, Yamaguchi H, Kimura M, Ohnuma H, Eguchi H, Igarashi M,

Manaka H, Kato T. Increase in serum ceruloplasmin with aging is not observed in type 2 diabetes. Endocrine journal. 2000;47(3):215-9.
26. King CM, Bristow-Craig HE, Gillespie ES, Barnett YA. In vivo antioxidant status, DNA damage, mutation and DNA repair capacity in cultured lymphocytes from healthy 75-to 80-year-old humans. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 1997;377(1):137-47.
27. Virginia B.C. Junqueira, Silvia BM., Barros, Sandra S. Chan, et al. Ageing and oxidative stress. Molecular Aspects of Medicine, 2004;25:5-16.
28. Everitt AV, Hilmer SN, Brand-Miller JC, Jamieson HA, Truswell AS, Sharma AP, Mason RS, Morris BJ, Le Couteur DG. Dietary approaches that delay age-related diseases. Clinical interventions in aging. 2006;1(1):11.

Cite this article as: Fasna KA, Geetha N, Maliekkal J. Oxidative stress in ageing. Int J Res Med Sci 2017;5:4826-31.