

# A Study on Effect of Ageing on the Levels of Total Antioxidant and Lipid Peroxidation

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## ABSTRACT

**Introduction:** Aging has raised significant social and health concern worldwide. Aging can be viewed as a process of irreversible cell injuries associated with the accumulation of oxygen derived free-radicals. The present study was undertaken to evaluate the changes that occur due to ageing on the levels of Total antioxidant concentration and lipid peroxidation.

**Material and methods:** Fifty healthy non-smoking elderly individuals without major illness belongs to the age group of >60years and fifty non-smoking young adults of age group of 18-40 years and who were willing to take part in the study were included after their consent institutional ethical clearance. A 5ml of venous blood was collected, serum was separated and used for the analysis of total antioxidant capacity and Malondialdehyde level.

**Result:** The results of the present study showed a significant decrease ( $p < 0.05$ ) in TAC elderly individuals as compared to the young adults and a significant increase ( $p < 0.0001$ ) in the MDA levels in the elderly population in comparison with the young adult population.

**Conclusion:** It can be concluded from the study, that a significant decrease in the antioxidant level and increase in the level of oxidative stress was observed as the age increases. This direct towards considering the levels of these oxidative stress markers as an important marker for the ageing

**Keywords:** Lipid Peroxidation, Total Antioxidant Capacity, Elderly population

## INTRODUCTION

Aging has raised significant social and health concern worldwide<sup>1</sup>. Aging can be viewed as a process of irreversible cell injuries associated with the accumulation of oxygen derived free-radicals<sup>2</sup>. Among them any proposed theories, the free radical theory of ageing has been able to provide a theoretical framework that comprises both stochastic and genetic factors<sup>3-5</sup>. In its general form, it postulates that ageing is the result of free-radical mediated damage to molecules and tissues, which accumulates with advancing<sup>2-7</sup>. Studies have shown that there is a decline in the total antioxidant activity in elderly people and may have a crucial role in the development of pathophysiological changes of aging<sup>8</sup>. Lipid peroxidation is a free radical mediated oxidative degradation of the lipids resulting in cell damage. Malondialdehyde (MDA) is a major product of free radical attack to membrane polyunsaturated fatty acids and it is, probably, the most widely used indicator of lipid peroxidation which is also considered as a biomarker of aging<sup>9,10</sup>. So, the present study was done to evaluate the changes that occur due to ageing on the levels of Total antioxidant concentration and lipid peroxidation.

## MATERIAL AND METHODS

The present study was carried out in Central Research Laboratory, K.S. Hegde Medical Academy, Nitte University, Deralakatte, Mangalore after the clearance obtained from the Institutional Ethical Committee. Written informed consent was taken from subjects taking part in the study after explaining the details of the study. The study population comprises of 50 healthy non-smoking elderly individuals without major illness belongs to the age group of >60years and 50 non-smoking young adults of age group of 18-40 years and who were willing to take part in the study were included. Subjects with the history of chronic illness like hypertension, diabetes, malignancy and also smokers, alcoholics and patients under any nutritional supplements etc. were excluded from the study.

A 5ml of venous blood was collected using disposable syringes, centrifuged and serum was separated. The collected serum was stored at  $-20^{\circ}\text{C}$  until analysis of total antioxidant capacity and Malondialdehyde level.

**Estimation of the total antioxidant capacity by phosphomolybdenum method<sup>11</sup>:** This quantitative assay is based on the conversion of Molybdenum (MoVI) by reducing agents like antioxidants to molybdenum (MoV), which further reacts with phosphate under acidic pH resulting in the formation of a green colored complex, the intensity of which can be read spectrophotometrically at 695nm.

**Estimation of Malondialdehyde (MDA) by Beuge Method<sup>12</sup>:** MDA formed by the breakdown of poly unsaturated fatty acids (PUFA) serves as a convenient index to determine the extent of lipid peroxidation. It reacts with TBA to give a pink color which is read at 535nm.

## STATISTICAL ANALYSIS

The data on TAC and MDA values were expressed as Mean  $\pm$  S.D. The data between the young and elderly population was compared using ANOVA. P value less than 0.05 was considered the level of significance.

## RESULTS

The TAC level in the elderly individuals was  $1.160 \pm$

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Parameter	Age group	Mean $\pm$ SD	95% Confidence interval	P- value
TAC	Age below 30	1.430 $\pm$ 0.0914	-0.4819 to -0.05852	*
	Age from 31-50	1.160 $\pm$ 0.061		

\* indicates p- value <0.05.

**Table-1:** Levels of Total Antioxidant Capacity (TAC) in subjects belongs to different age groups.

Parameter	Age group	Mean $\pm$ SD	95% Confidence interval	P- value
MDA	Age below 30	0.963 $\pm$ 0.0484	0.7908 to 1.173	***
	Age from 31-50	1.945 $\pm$ 0.0816		

\*\*\* indicates p- value < 0.0001

**Table-2:** Levels of Malondialdehyde(MDA) in subjects belongs to different age groups.

0.061mM/L and in young adults was 1.430  $\pm$  0.0914 mM/L. On analysis this showed a significant decrease (p<0.05) in elderly individuals as compared to the young adults (table-1). The MDA level in the elderly population was 1.945  $\pm$  0.0816  $\mu$ M/L and in young adult population was 0.963  $\pm$  0.0484  $\mu$ M/L. On analysis this showed a significant increase (p<0.0001) in the MDA levels in the elderly population in comparison with the young adult population (table-2).

## DISCUSSION

Oxidative stress (OS) can be defined as an imbalance between the production of free radicals and the body's antioxidant defense. OS can cause damages to biomolecules such as lipids, proteins and DNA and is involved in the pathogenesis of many diseases. Lipid peroxidation refers to the oxidative degradation of lipids which forming malondialdehyde (MDA) that can be used as a biomarker to measure the level of OS in an organism. Antioxidants are substances that play a major role in preventing the formation and in scavenging of free radicals. In early 1990s, a method for the measurement of total antioxidant capacity (TAC) was developed. The major advantage of this test is to measure the antioxidant capacity of all antioxidants in a biological sample and not just the antioxidant capacity of an individual compound. Study by Carlos Kusano et al., TAC could be useful to evaluate nutritional interventions with TAC-rich foods on disease risk and prevention, including anti-aging strategies.<sup>7</sup>

According to the study conducted by Victor et al., 62% of the elderly subjects of age group between 60 to 69 years presented low levels of total antioxidant levels measured by a colorimetric method.<sup>1,2</sup> Another study by Andriollo-Sanchez et al.,<sup>13</sup> was done to compare the level of oxidative stress parameters between middle-aged and older subjects, suggest a progressive and slow decline of antioxidant status in healthy free-living older subjects and underline the impact on lifestyle factors in successful ageing. The present study results correlate with the above-mentioned studies. Nucci et al.<sup>14</sup> carried out a comparative study to evaluate the levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) in the blood an aqueous humor of glaucomatous and non-glaucomatous patients. In conclusion, they were of the opinion that oxidative stress and decreased antioxidant defenses are involved in the pathogenesis of glaucoma. The study results suggest an increase in the level of lipid peroxidation in elderly population when compared with the younger population.

## CONCLUSION

The results of the present study conclude an increase in

the level of lipid peroxidation and decreases in the Total antioxidant concentration was observed in the healthy non-smoking elderly individuals when compared with the healthy non-smoking individuals of the age group below 30 years. This clearly shows there is significant decrease in the antioxidant level and increase in the level of oxidative stress was observed as the age increases. This also will direct towards considering the levels of these oxidative stress markers as an important marker for the ageing

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