

## EVALUATION OF OXIDATIVE STRESS BETWEEN MIDDLE AGED AND ELDERLY POPULATION OF KOLKATA AND SUBURBS

Sayari Banerjee\* and Chinmoy Ghosh

Department of Biochemistry, Medical College & Hospital, Kolkata, West Bengal, India.

### ABSTRACT

It has been demonstrated by numerous studies that there is a slow decline of antioxidant status in older subjects with simultaneous increase in oxidative stress which is associated with increased production and accumulation of reactive oxygen species in elderly people. The present hospital based non-interventional cross-sectional study was designed to compare age-related oxidative stress as well as antioxidant status in middle aged (35-55y) and older (>60y) of Kolkata and its suburbs by measuring serum Thiobarbituric acid reactive substances, serum protein carbonylation, vis-à-vis antioxidant defence with estimation of plasma superoxide dismutase. The Measurement of concentration of protein carbonylation and Thiobarbituric acid reactive substances in serum helps to identify the impact of age related oxidative stress. Superoxide dismutase is an antioxidant enzymes that catalyzes the dismutation of superoxide into oxygen and peroxide. Estimation of superoxide dismutase in plasma is important to evaluate the antioxidant status. Effect of age related oxidative stress and antioxidant parameters were investigated in 40 elderly people >60years attending geriatric department of the hospital for counseling (but otherwise healthy) and also in 40 middle aged persons (35-55y). On the basis of results obtained from this study it was evident that serum protein carbonylation and concentration of serum Thiobarbituric acid reactive substances were significantly higher in older subjects. It has been observed that enzymatic antioxidant serum Superoxide dismutase was significantly lower in elderly age group than middle aged group. The present study provide some important information regarding age related oxidative stress in healthy elderly population compared to middle aged subjects.

**Keywords:** aging, oxidative stress, antioxidant, elderly, middle-aged.

### INTRODUCTION

There is no dearth of evidence to show that aging might be caused by deleterious and cumulative effects of reactive oxygen species generated throughout the life span. ROS may cause serious and deleterious damages to all types of biological molecules like protein, lipids or DNA etc. Production of oxygen species such as free radicals namely hydroxyl radical, superoxide radical, oxygen singlet etc and peroxides is considered as particularly destructive aspect of oxidative stress. The reactive species produced by oxidative

stress can cause direct damage to DNA causing mutation and it may promote proliferation, invasiveness and metastasis. Protein carbonylation is a type of protein oxidation. Among the diseases in which high levels of protein carbonyl (CO) groups have been observed include Alzheimer's disease, rheumatoid arthritis, diabetes, sepsis, chronic renal failure and respiratory distress syndrome<sup>1,2</sup>. The quantification of protein carbonyl groups in peripheral blood is widely used to measure the extent of oxidative modification. Oxidative

degradation of lipids is referred to as lipid peroxidation. Lipid peroxidation is a process in which free radicals take away electrons from membrane lipid, resulting in cell membrane damage. Markers of lipid peroxidation have been verified in many diseases such as ischemic heart disease, diabetes and neurodegenerative disease. End product of lipid peroxidation was estimated by measuring serum Thiobarbituric Acid Reactive Substances (TBARS). Superoxide dismutase (SOD) is an important factor in limiting oxygen toxicity<sup>3</sup>, it is one of the best studied metalloenzymes in human biochemistry.

The present hospital based non-interventional cross-sectional study aims at finding out association between oxidative stress with antioxidant defence in relation to age. Justification and relevance of our proposed research work is based on the fact that although studies in healthy elderly population in developed countries have shown that oxidative stress may lead to an accelerated aging and higher incidence of oxidative diseases but there is lack of significant works in this area in developing countries like India specially in eastern region

## MATERIALS AND METHODS

### STUDY DESIGN

Hospital based, non-interventional cross sectional study. Random sampling was done and control was not required in this study. Clearance was obtained from the institutional ethical committee. Written informed consent as per local language was taken from individuals taking part in this study after explaining the details of the study.

### SUBJECTS

A total of 40 elderly but otherwise healthy volunteers aged >60 yrs attending the geriatric OPD of the hospital for the counseling constituted the elderly group and 40 middle aged volunteers aged (35-55 y) constituted the middle aged group.

### EXCLUSION CRITERIA

History of chronic diseases like hypertension, diabetes, rheumatoid arthritis, any neuropsychiatric disorder like Parkinson's disease, motor neuron disease, chronic depression, any endocrinal abnormality, malignancy, smokers, drinkers and patient under any nutritional supplement etc.

### MATERIALS USED

Trichloroacetic Acid (TCA) solution 2.5 MHC solution 0.5% TBA solution

n-Butanol, Stock Standard Solution (1mM 1,1,3,3-TetraethoxyPropane), 2,4-DNPH (10 mmol/L), HCL (2 mol/L), Protein washing solution (ethanol : ethyl acetate =1:1), Protein dissolving solution (2 gm SDS & 50mg EDTA in 100 ml of 80 mmol /L phosphate buffer, pH-8), Sodium pyrophosphate buffer (0.025M, pH 8.3), PMS (phenazinemethosulfate - 186 micro M) sol, NBT (nitrobluetetrazolium 300 micro M), NADH sol -780 micro M, Glacial acetic acid and other chemicals used in the study are of analytical grade.

## ANALYTICAL METHODS

**Collection of sample** -10 ml of venous blood was collected aseptically from the individuals. For accurate comparison, fasting normal samples were obtained. 2ml blood collected in EDTA vial and rest was collected in container having no anticoagulant

### Assay of Thiobarbituric acid reactive substances (TBARS)

: Serum level of TBARS was measured by method of Dahle, LK., et al<sup>4</sup> (1962). 0.5ml serum and 2.5 ml TCA were kept for 10 mins at room temperature. 2.5 ml of 2.5 mol HCL was added with constant stirring and to this 3.5 ml of TBA was added and incubated in boiling water bath for 30 mins. After cooling Butanol was added and vortexed for 1min. It was centrifuged at 3000 rpm for 10 mins and supernatant (coloured MDA-TBA complex) was measured at 532 nm in a spectrophotometer.

### Assay of serum protein carbonylation

: Serum protein carbonylation was evaluated by Levine's method<sup>5</sup> (1990). Serum was treated with 10% TCA and 2,4 dinitrophenylhydrazine (DNPH) was added to the precipitated protein after removal of impurities. DNPH reacted with carbonylated protein and converted into 2,4 dinitrophenylhydrazone which has specific colour which was measured spectrophotometrically at 370 nm wave length. Concentration of carbonylated protein was calculated as per Levine's method.

### Assay of Superoxide dismutase (SOD)

: Plasma SOD was measured by the method of Kakkaret al<sup>6</sup> (1989). 1.35ml of double distilled water, 50µl of plasma, 1.2 ml of sodium pyrophosphate buffer (pH 8.3), 0.1ml of PMS and .3ml of NBT was mixed. 0.2ml of NADH solution was added to it to initiate the reaction. After incubation at 39 degree for 90 seconds the reaction was terminated by

adding 1ml of glacial acetic acid.4ml of n-butanol was added and mixed vigorously by vortexing.The mixture was centrifuged at 4000 rpm for 10 minutes and the absorbance of upper butanol layer was measured at 560nm.For the comparison ,corresponding blank was prepared in the same way except addition of plasma.One unit of SOD was defined as the amount of enzyme that inhibited the rate of reaction by 50% under specified conditions.

### Statistical methods

Healthy human volunteers were selected according to pre set inclusion and exclusion criteria.Total 80 person fulfilled the inclusion criteria.Data analysis was performed using SPSS (version17) and Statistica version 6( Tulsa, Oklahoma:statsoft Inc,20001) .Values were expressed as Mean  $\pm$  SEM .Statistically significant difference was determined with the student's Independent t- test (two-tailed ).The  $P < 0.05$  was considered significant.Correlation study was done.

### RESULTS AND DISCUSSION

In this study we got 2 group of population-**GROUP A**) Middle aged population (35-55y))&**GROUP B** ) Elderly population(>60y).All variables are normally distributed by Kolmogorov-Smirnov goodness-of-fit test.

TABLE 1 shows that Serum protein carbonylation is significantly higher in elderly population( $1.89 \pm 0.409$  ) than middle aged ( $1.36 \pm 0.426$ ).Serum TBARS is significantly higher ( $12.57 \pm 0.490$ ) in elderly than middle aged population ( $8.83 \pm 0.467$ ).Plasma SOD level is significantly lower ( $3.98 \pm 0.475$  ) in elderly than middle aged population ( $4.42 \pm 0.431$ ).

From TABLE 2 it can be demonstrated that .It is evident that serum TBARS is significantly higher in elderly population than middle aged.( $p = 0.000$ ).It is seen that serum protein carbonylation is significantly higher in elderly than middle aged ( $p = 0.000$ )Superoxide dismutase activity is significantly lower in older age group.( $p = 0.000$ ).

Table 3 represents results of correlation analysis .Correlation analysis shows There is significant positive correlation( $r = 0.92$ ) between age and serum TBARS concentration. There is good correlation ( $r = 0.53$ ) between age and serum protein carbonylation. Plasma SOD negatively correlated ( $r = -0.39$ ) with age.

Fig 1 showed significant positive correlation between age and TBARS.

Fig 2 shows significant positive correlation between age and serum protein carbonylation.

Oxidative stress reflects an imbalance between the systemic manifestations of reactive oxygen species and a biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage.It has been demonstrated by numerous studies that aging is the sum of all free radical reaction throughout all cells and tissues or that they are atleast a major contributor to it.

ROS may cause serious and deleterious damages to all types of biological molecules like protein,lipids or DNA but proteins are possibly the most immediate vehicle of inflicting on cells.It has been generally demonstrated by several studies that protein peroxidation increases with progression of age and protein carbonylation is an early determinant of oxidative stress.

In this experimental study we have also noticed that serum protein carbonylation is significantly higher ( $p = 0.000$ ) in older subjects than middle aged, which signifies noticeable increase in oxidative stressor in older subjects than young adults. We have noticed significant positive correlation (  $r = 0.53$ ) between age and protein carbonylation.In the opinion of Kaspoglu and Ozben protein oxidation is generally reported to increase during aging.<sup>7</sup> These findings are in agreement with the observation of Bureau et al which he obtained from his study with women group<sup>8</sup>.Mezatti et al<sup>9</sup> showed that plasma protein peroxidation products is higher in elderly than in younger subjects. Saha A etal<sup>10</sup> observed an enhanced oxidative stress by an increased protein carbonyl content both in plasma and in hemolysate of the diseased samples in type 2 diabetes and diabetes associated cardiovascular disease patients in their Kolkata based studies, but according to Isabella-Dalle-Done<sup>2</sup>etal what relationship might be among high level of carbonyl( CO) groups, oxidative stress and disease are still uncertain .

Another common approach to estimate oxidative stress in vivo is to measure the end products of lipid peroxidation.The most widely used index is plasma malondialdehyde (MDA) ,which is measured by thiobarbituric acid reacting substances (TBARS) assay.TBARS are formed as a byproduct of lipid peroxidation (i.e.degradation effect ) which can be detected by TBARS assay using thiobarbituric acid as reagent.The present study included measurement of the concentration of TBARS for quantification of the endproducts of lipid peroxidation. In so far as the present study is concerned it is clearly evident

from the results obtained from our assessment that serum TBARS is significantly higher ( $p=0.000$ ) in elderly population than middle aged population and there is a positive correlation ( $r=0.92$ ) between age and TBARS concentration.

Chan et al<sup>11</sup> in their Sao Paulo oxidative and aging study reported that plasma concentration of TBARS increased significantly in individuals over 50 yrs age as compared with younger group.

Mezzetti et al<sup>9</sup> & Block et al<sup>12</sup>, referred to increased in lipid peroxidation products but they too did not mention any direct correlation between age and TBARS level. Andrela-Sanchez et al<sup>13</sup> in their work on European population postulated that TBARS production is dependant on consumption of polyunsaturated fatty acid. So it may be presumed that variation in results obtained by different investigators belonging to different regions & parts of the world may have some relation to lifestyle and food habits of elderly population and age group of enrolled participants of concerned study. Gill et al<sup>14</sup> postulated that the balance of oxidant and antioxidant systems in plasma shifts in favour of accelerated oxidation of protein and lipid (carbonyl & MDA) during aging. Rizvi SI and Maurya Rk<sup>15</sup> observed a higher oxidative stress (increased MDA) in Indian population compared to values reported for European subjects. Saaswati M et al<sup>16</sup> demonstrated an increased level of oxidative stress marker and altered lipid profile in urban diabetics (type 2) and healthy controls corresponding to respective rural population suggesting the effect of urbanisation and impact of different life style.

The most important enzymatic antioxidant is superoxide dismutase (Cu-Zn SOD) which catalyzes conversion of superoxide anions into  $H_2O_2$  which is deactivated to  $H_2O$  by catalase. In the present study it has been observed that Cu-Zn SOD activity is significantly lower ( $p=0.000$ ) in older age group and SOD has a negative correlation ( $r=-0.39$ ) with age. Similar to our observation Anderson et al<sup>17</sup> observed an age related decrease in Cu-Zn SOD activity. Guemouri et al<sup>18</sup> noted that SOD activities appear rather stable in adults less than 65 years old but the decrease for most enzymes in the elderly. Marjini A<sup>19</sup> in their studies with regard to age related alterations of plasma lipid peroxidation and erythrocyte SOD in different age group of Gorgan city of Iran observed that plasma lipid peroxidation (MDA) significantly increase with aging. They have also observed that erythrocyte SOD activity significantly decreased with aging. SOD may play an important role in

determining individual risk of developing certain diseases such as cancer or atherosclerosis etc. Besides constitutional individual differences in gene expression, antioxidative enzyme activities apparently depend on variations in life style and environmental factor.

However so far as oxidative stress and antioxidants are concerned, observation of the present study fits fairly well with hypothesis of free radical theory of aging<sup>20</sup>. More over, the comparison between middle aged and older subjects in relation to oxidative stress parameters suggest a progressive and slow decline of antioxidant status in healthy free living older subjects underlining the impact of life style factors in aging. Since fundamental mechanism of age related oxidative stress and role of antioxidants is not clearly understood, this subject is still existing as a contentious issue among the scientists working on this subject matter. In addition, analytical differences between laboratories make it difficult to compare the results obtained in different studies. In our endeavour to study the effect of certain parameters of oxidative stress and antioxidant status between middle aged and elderly population, significant increase in oxidative stress factors and simultaneous decline in antioxidant status was found.

## CONCLUSION

Data obtained from the present study provide some important information regarding age related oxidative stress in healthy elderly population compared to middle aged subjects. In this present study the result shows that serum protein carbonylation is significantly higher ( $p<0.000$ ) in older subjects than middle aged and there is a positive correlation ( $r=0.53$ ) between age and serum protein carbonylation. Serum TBARS is significantly higher ( $p=0.000$ ) in elderly than middle aged and positively correlated ( $r=0.92$ ) with age. serum SOD is significantly lower ( $p<0.000$ ) in older age group and SOD is negatively correlated ( $r=-0.39$ ) with age. These findings highlight that free living healthy elderly subjects of Kolkata and suburbs are exposed to significant oxidative stress. Moreover, on the basis of a comparison between middle aged and elderly subjects in relation to oxidative stress parameters as well as antioxidant activities, it can be concluded, that there is a definitive decline of antioxidant status in healthy free living older subjects when compared to middle aged subjects.

Table 1

Parameters	Group A (Middle Aged) No of cases(n) = 40	Group B (Elderly) No of cases (n)=40
Serum TBARS ( $\mu\text{mol/L}$ ) Mean $\pm$ SD (SEM)	8.830 $\pm$ 0.466 (0.0783)	12.569 $\pm$ 0.490 (0.0775)
Serum protein carbonylation (nmol/mg of serum protein) Mean $\pm$ SD (SEM)	1.357 $\pm$ 0.426 (0.0674)	1.887 $\pm$ 0.408 (0.0646)
Plasma SOD (unit/ml) Mean $\pm$ SD (SEM)	4.417 $\pm$ 0.430 (0.0681)	3.976 $\pm$ 0.475 (.0751)

Table 2: Test of significance (Independent t- test-2 tailed) of different parameters between middle aged and elderly

Parameters	t-score	Significance (2-tailed)	95% confidence interval of difference	
			Upper	Lower
Serum TBARS	-34.930	0.000	-3.526	-3.952
Serum protein carbonylation	-5.672	0.000	-.343	-.715
Super oxide dismutase	19.854	0.000	.6428	.2391

Table 3: Correlations between numerical variables without categorizing age groups – Pearson's correlation coefficient r. Correlations in bold are significant at the level of  $p < 0.05$ .

Age	TBARS	ProtCarbonyl	SOD	
Age	1.00	<b>0.92</b>	<b>0.53</b>	<b>-0.39</b>
TBARS	<b>0.92</b>	1.00	0.57	-0.40
ProtCarbonyl	<b>0.53</b>	0.57	1.00	-0.29
SOD	-0.39	-0.40	-0.29	1.00

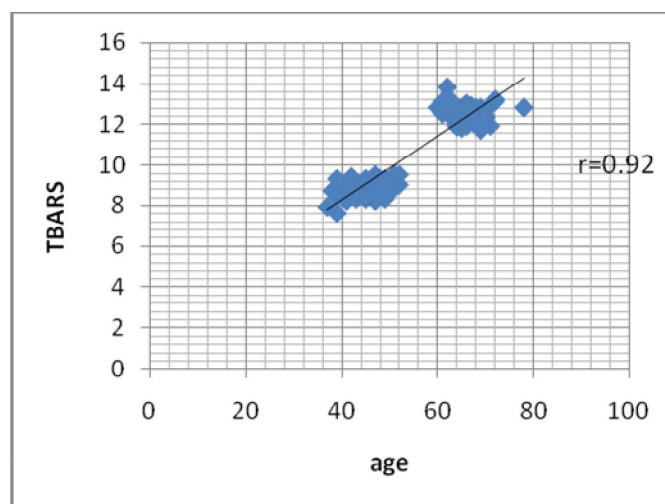


Fig. 1: Scatter chart showing correlation between age and TBARS

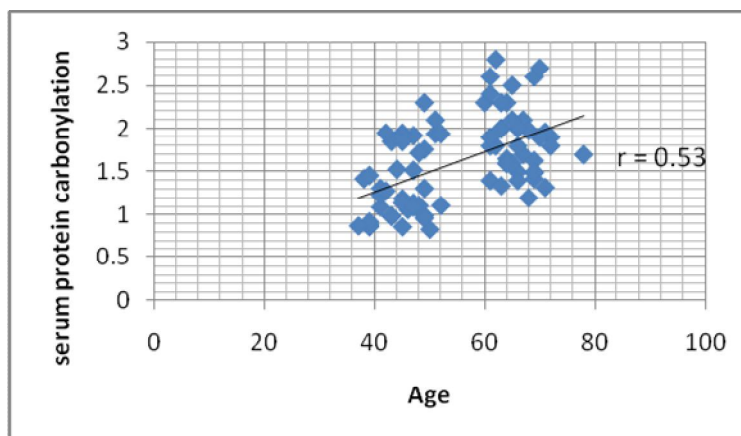


Fig. 2: Scatter chart showing correlation between age and serum protein carbonylation

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