Original Article

Oxidative Stress Among the Middle Aged and Elderly Population: A Comparative Study

Jyoti Sinha, Tanmay Saha✉, Subir Kumar Das

ABSTRACT

Background: Ageing and death has been described as a result of interplay between several programmed or non-programmed factors. Mitochondrial DNA damage increases with advancement of age resulting increased Reactive oxygen species production and oxidative stress. Other than DNA oxidative stress affects the membrane of the cell and intracellular organelle. by alteration of membrane proteins and lipids. Cumulative effects of protein, lipid and DNA damage with progression of age might be a major cause of ageing of a living organism.

Material & Method: A total 72 study participants were distributed equally in two groups depending on their age. The groups were A (35-55 Yrs.) and B (>60 Yrs.) with 36 participants in each group. Serum thiobarbituric acid reactive substances, Protein Carbonyl, Superoxide dismutase and α-tocopherol were measured in 12 hrs fasting venous blood sample of both the groups.

Result: Thiobarbituric acid reactive substances and Protein Carbonyl were found to be significantly increased among the elderly age group ($p < 0.001$). On the other hand, α-tocopherol ($p < 0.001$) and Superoxide dismutase ($p < 0.05$) were significantly decreased in the elderly study group. When all 72 participants in both group were considered together, it was found that TBARS and Protein carbonyl were found significant positive correlation with age ($r = 0.90, p < 0.01$) and ($r = 0.44, p < 0.01$) respectively. On the other hand α-tocopherol and SOD were found to have significant negative correlation with age ($r = -0.88, p < 0.01$) and ($r = -0.27, p < 0.05$) respectively.

Conclusion: Elderly population experiences more oxidative stress compared to their middle aged counterparts.

Key words: Oxidative stress; protein Carbonyl; superoxide dismutase; thiobarbituric acid reactive substances; α-tocopherol

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INTRODUCTION

Ageing has been described as a result of interplay between the several programmed or non-programmed factors.[1] Recent studies have established that mitochondrial DNA damage increases with advancement of age[2,3] which clearly supports the mitochondrial theory of ageing.[4] Unfortunately, despite of several theories and hypotheses, the mechanism still has remained unexplained to a larger extent. However, the role of free radicals and thus oxidative stress in ageing has been accepted unanimously. Oxidative stress theory of ageing has stated that increased reactive oxygen species (ROS) level results in alteration of physiological processes that ultimately leads to ageing and death.[5]

In a biological system, level of oxidative stress is the result of balance between production of ROS and status of antioxidants.[6] Effects of the oxidative stress on the same biological system depend upon the magnitude of such changes. Depending upon the degree of oxidative stress, the cell may undergo apoptosis, necrosis and even cell death.[7] In the event of oxidative stress, ROS are generated including superoxide, peroxide and hydroperoxide free radicals which further react with transition metals or other redox cycling compounds like quinines. Such crosstalk between the free radicals and other compounds may convert them into more detrimental form.[8]

Free radicals are well known agents for DNA damage and interestingly such damage is quite similar with the DNA damage induced by ionizing radiation.[9] Both nuclear and mitochondrial DNA may undergo single strand break, double strand break, loss of H^+ from OH group of ribose sugar or modification of bases. Other than DNA oxidative stress and resultant ROS also affects the membrane of the cell and intracellular organelle by alteration of membrane proteins and lipids. ROS damage to proteins is manifested as carbonylation of protein, appearance of hydroperoxy group and formation of chlorine and nitrogen derivatives.

The Peroxyl radical, hydroxyl radical, alkalyl radical causes damage to lipids. MDA is the major aldehyde produced from the peroxidation of biological membrane. It is produced only when peroxidation occurs in fatty acids containing three or more double bonds.[10] The cumulative effects of accumulation of oxidative stress and free radical generation and resultant protein, lipid and DNA damage with progression of age might be a major cause of ageing and death of a living organism.[11]

Increased oxidative stress has been reported among the elderly subjects. Increased production of free radicals and decreased antioxidant concentration might be the major causes. However, alteration in antioxidant defense along with progression of age among the elderly population has not yet been ascertained and thus there is a major lacuna in information regarding their evolution from young adult to older individual.[12] Hence the present study was designed to compare the age related oxidative stress and antioxidant defense between middle aged (35-55 yrs.) and older subjects (>60 yrs.) of Kalyani town of West Bengal and nearby areas by estimating the serum protein carbonylation and TBARS as a marker of protein and lipid peroxidation and also by assessing plasma SOD, serum α-tocopherol concentration.

MATERIALS AND METHODS

The present hospital based, descriptive, cross sectional study was undertaken at the Department of Biochemistry, College of Medicine and JNM Hospital, WBUHS, Kalyani, Nadia, West Bengal. A total 72 study volunteers were equally distributed in two groups namely group A (35-55 years)

and group B (> 60 years) each comprised of 36 participants. Study participants were selected randomly irrespective of sex from the population attended the hospital for routine check-up. Individuals with history of smoking, alcoholism, neuropsychiatric disorder or on nutritional supplements or receiving treatment for any other chronic illness were excluded from the study. As most of the metabolic processes and thus biochemical parameters are altered in pregnancy, pregnant women also were not included in the study. The study was approved by the Institutional Ethics Committee (Reference No. F-24/PR/COMJNMH/IEC/18/1250). Written consents were collected from 72 study participants who fulfilled the inclusion criteria. Depending upon age distribution they were equally distributed in two groups namely group A (35-55 years) and group B (>60 years).

After 12 hrs of fasting, 6 ml venous blood was collected from each participants. Samples were divided into two parts. 4 ml blood was transferred in vials without any preservative or anticoagulant and 2 ml in EDTA vials. Following collection the samples were centrifuged to collect serum for analysis of TBARS, Protein carbonylation, SOD and Tocopherol content. Analysis of the samples was performed immediately after collection.

Quantitative of analytes was performed using UV-VIS Spectrophotometer 117 (Spectronics). Following methods were followed for estimations:

Serum TBARS - Dahle, LK.et al,[13] absorbance taken at 532 nm against butanol as blank; protein carbonylation - Levine's method,[14] absorbance taken at 370 nm; Alpha tocopherol - Baker and Flank method,[15] absorbance taken at 520 nm; SOD - Kakkar et al,[16] absorbance taken at 560 nm.

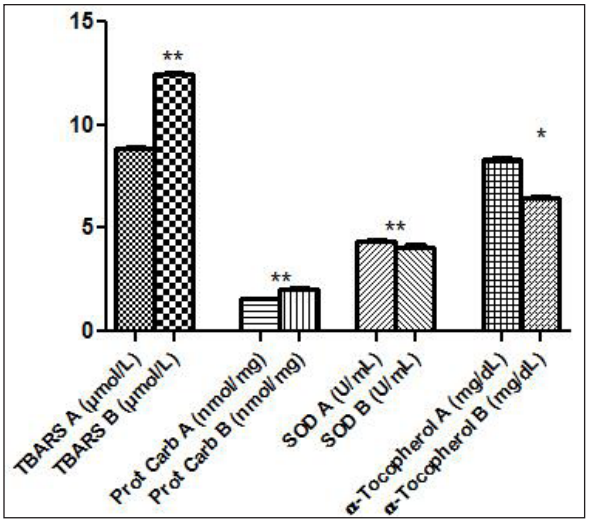
Statistical analysis:

TBARS, Protein carbonylation, alpha tocopherol and SOD were measured in both the groups and results are expressed in Mean ± Standard Error (SE) using SPSS statistical software version 23. Graphical representation of data was prepared using Graphpad Prism version 5.0. Student's independent t-test was performed to find out the significance of mean difference of all parameters in group A and B.

RESULTS

Group A and group B each had 36 participants. Mean age were 45.48 ± 0.78 and 67.36 ± 0.87 years respectively. TBARS and Protein Carbonyl were found to be significantly increased among the elderly age group when compared to middle aged study group ($p < 0.001$). Group A had TBARS (8.83 ± 0.09 µmol/L) and Protein Carbonyl (1.43 ± 0.07 nmol/mg) respectively, while the group B had TBARS (12.44 ± 0.08 µmol/L), Protein Carbonyl (2.03 ± 0.07 nmol/mg) respectively.

As shown in table 1 and figure 1, α-tocopherol and SOD were significantly



Where ** is significant at the level of 0.001 & * is significant at the level of 0.05

Figure 1: Oxidative stress parameters in middle age and elderly age groups,

decreased in the elderly study group (Group B). The levels of α- tocopherol were 8.36 ± 0.06 mg/L in group A and 4.1 ± 0.07 mg/L in group B respectively ($p < 0.001$) and SOD showed 6.43 ± 0.06 U/ml and 4.35 ± 0.08 U/ml in group A and group B respectively ($p < 0.05$).

Next, we determined the correlation of oxidative stress markers with age. For this, Pearson's correlation (two tailed) was performed was and found that TBARS and Protein carbonyl have significant positive correlation with age ($r = 0.90$, $p < 0.01$) and ($r = 0.44$, $p < 0.01$) respectively (Figure 2). On the other hand α-tocopherol and SOD were found to have significant negative correlation with age ($r = –0.88$, $p < 0.01$) and ($r = –0.27$, $p < 0.05$) respectively (Figure 2).

Table 1: Oxidative stress parameters in middle age and elderly age groups

Parameters	Group A (n = 36)	Group B (n = 36)	P value
Age (yrs)	45.48 ± 0.78	67.36 ± 0.87	< 0.001
TBARS (µmol/L)	8.83 ± 0.09	12.44 ± 0.08	< 0.001
Protein Carbonyl (nmol/mg)	1.43 ± 0.07	2.03 ± 0.07	< 0.001
α-Tocopherol (mg/L)	8.36 ± 0.06	4.1 ± 0.07	< 0.001
Superoxide Dismutase (U/ml)	6.43 ± 0.06	4.35 ± 0.08	0.022

All values are expressed as mean ± SE. *p < 0.05 was considered significant

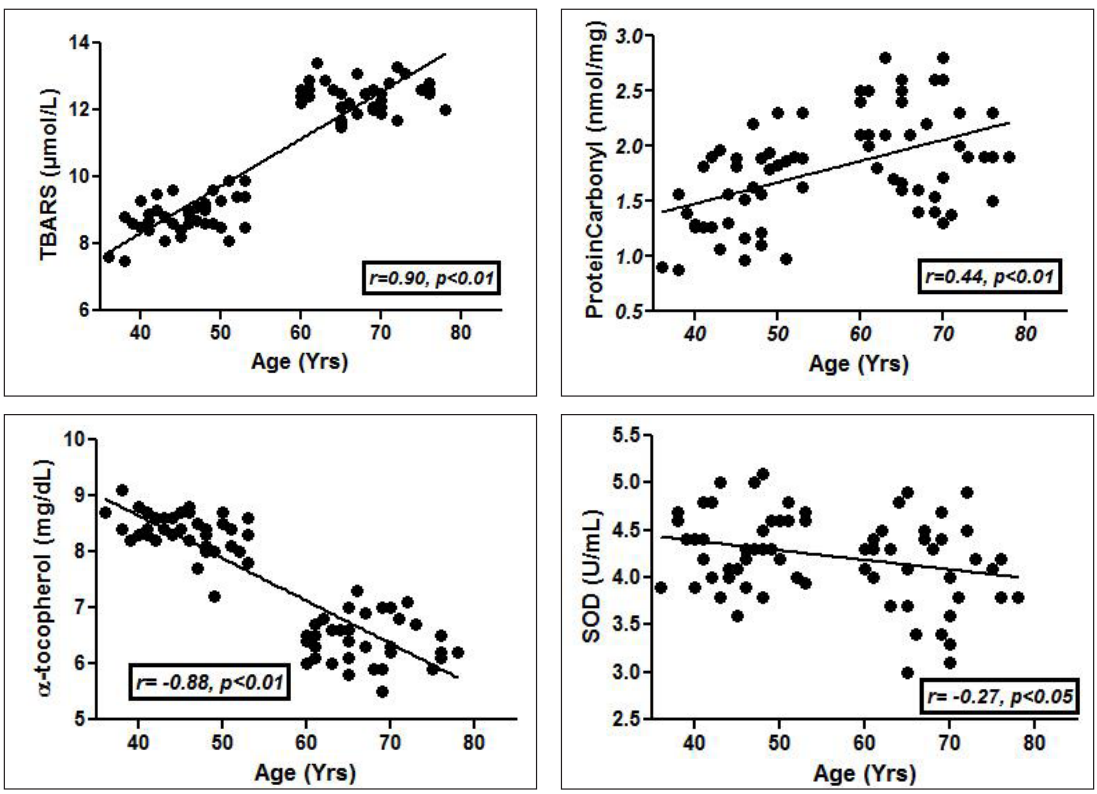


Figure 2: Correlation between age and oxidative stress parameters

DISCUSSION

Progression of age and age related mortality is inevitable for a living organism. Remarkable increase in life expectancy probably is the greatest achievement in the past century for human civilization. In last century there was hardly any country with life expectancy more than 50 years while currently many are with 80 years of life expectancy.[17] Hence, with time the proportion of elderly age group in society is gradually increasing significantly and trend is a continuous process. Currently, while significant reduction in the prevalence of some physical and mental disabilities have been achieved, but at the same time the prevalence of other geriatric diseases have been increased, largely due to increased expected life span. To reduce such age related mortality and to increase the life expectancy further, the major requirement is delaying of ageing. For that it is of great biomedical importance to understand the underlying causes and the changes that accompany it which triggers of aging.

ROS such as hydroxyl radicals and superoxide etc., are highly reactive and most often participate in chain reactions which multiply their effect. Most importantly their production is a continuous process as byproducts of a complex network of redox reactions taking place in the mitochondrial electron transport chain. Mitochondria occupy a central place in many theories of aging. This importance of mitochondria can be attributed to various factors, but most significant is the role of mitochondria as a major source of ROS in the cell[1].

It has been shown by several studies that protein peroxidation increases with progression of age and protein carbonylation is an early determinant of oxidative stress.[18] ROS may cause deleterious damage to all types of biological molecules like protein, lipids or DNA. ROS induced membrane damage is a well known fact.[19] Further ROS and reactive nitrogen species may react to form peroxinitrite which is a strong prooxidant that results in covalent modifications of proteins.[20] In our study we have found significantly higher protein carbonyls (CO) concentration among the elderly age group which can be the result of oxidative cleavages of the protein backbone, direct oxidation of amino acids or by binding aldehyde produced from lipid peroxidation.

Another popular approach to estimate oxidative stress is to measure the end products of lipid peroxidation. The most widely used parameter is plasma malondialdehyde (MDA), which is measured by thiobarbituric acid reactive substances (TBARS) assay.[13] Lipid peroxidation results in TBARS generation as byproduct (i.e. degradation effect) which can be detected by TBARS assay. In our study we found significantly higher serum TBARS concentration among the elderly population when compared to middle aged population which is in accordance with the earlier study.[21]

Both SOD and α-tocopherol are well known potent antioxidants. We found decreased levels of SOD and α-tocopherol in elderly age group which might be the result of increased consumption to combat increased ROS generation. Both of these findings reflect the increase of oxidative stress with advancement of age. Increased ROS generation can result in cell death by alteration of membrane channels and transporters.[22]

Our finding can be explained by age related decline of mitochondrial function and its reduced ability to prevent electron and proton leakage from electron transport chain (ETC). With progression of age, the functional alteration takes place in the components of ETC which are important components of mitochondrial decay.[23-25] Such alterations also involve the lipid environment of the membranes[26,27] and

the resultant alteration of the inner mitochondrial membrane finally may end up with decreased aerobic metabolism, increased generation of ROS and oxidative stress consequently. Increased ROS generation results in increased mitochondrial DNA damage and thus possibilities of mutations which further affects the IEC components. Thus progression of age, alteration of mitochondrial membrane physiology, increased ROS generation and mitochondrial DNA damage forms a vicious cycle.[28-30] There is enough evidence that destabilization in mitochondrial super-complexes are responsible for age related mitochondrial pathologies.[31]

Ubiquinone (CoQ) is highly lipid soluble mobile component of ETC and the major component of Q cycle which is responsible for scavenging free radicals and thus prevents ROS generation. After attaining 20 years of age CoQ decreases over time.[32] Studies have shown an age dependent reduction of CoQ level in plasma and different tissues. By the age of 80 yrs, there is 50% reduction of CoQ in myocardial tissue. Similarly, among the elderly population, the reduction of CoQ in brain has been confirmed by other studies.[32] Thus a decrease of CoQ in the scenario of poorly functioning inner mitochondrial membrane-ETC component super-complexes always puts elderly people under the risk of significant oxidative stress.

This study has some limitations. A larger sample size would provide truer picture. Secondly, a prospective study with before and after antioxidant supplementation and comparison of paired data might provide better conclusion on the role of oxidative stress during aging.

CONCLUSION

Our study has showed that with the progression of age, oxidative stress increases significantly which is reflected by increased oxidative stress parameters in serum like TBARS and Protein Carbonyl and also decreased serum antioxidant concentrations including SOD and α-tocopherol. It has also found that both serum TBARS and Protein Carbonyl correlate positively with age and serum SOD and α-tocopherol have significant negative correlation with age.

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Conflict of interest

There is no conflict of interest in the study.

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