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RESEARCH ARTICLE

LIPID PEROXIDATION AND ANTIOXIDANT STATUS: A COMPARISON BETWEEN MIDDLE AGED AND ELDERLY POPULATION

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ABSTRACT

Objective: In recent years a large body of experimental research indicates that oxidative stress and antioxidant defenses are related to processes such as aging and several diseases. Vitamins and antioxidant enzymes have a fundamental role in defending organisms from oxidative stress. The objective of present study was to compare age related oxidative stress in middle aged and older subjects.
Design: The present hospital based nonintervention cross sectional study was designed to evaluate age related oxidative stress and its impact in 40 middle aged (35-55y) and 40 older subjects (60y) by measuring Thiobarbituric Acid Reactive substances (TBARs) for lipid peroxidation vis-à-vis antioxidant defense with estimatimation of plasma superoxide dismutase (SOD) and serum alpha-tocopherol concentration .
Intervention: none
Results: On the basis of the data obtained from our study it was evident that concentration of Thiobarbituric Acid reactive substances were significantly higher in older subjects, whereas enzymatic antioxidant serum superoxide dismutase and free radical scavenging alpha-tocopherol (vitamin E) were significantly lower in elderly age group than middle aged subjects.
Conclusion: The present study provides some important information regarding age related oxidative stress in elderly population compared to middle aged subjects. Lipid peroxidation which refers to oxidative degradation of lipids and acts as an oxidative stressor in the organism is significantly higher in elderly subjects but in so far as antioxidants are concerned we observe that they are significantly lower in elderly than middle aged subjects.
Keywords: aging, antioxidant, lipid peroxidation, oxidative stress

INTRODUCTION

Numerous studies have demonstrated that elderly adults have lower antioxidant and higher lipid peroxide .The aging process has been described by various theories. There is enough experimental evidence in support of the idea that aging is sum of all free radical reactions generated throughout lifespan. Oxidative stress is essentially an explicit manifestation of an imbalance between systemic production of reactive oxygen species (ROS) and a biological system's competence to readily detoxify the harmful reactive intermediates or its ability to repair the resulting damage[1]. Misbalance in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals which can cause damage to all components of cells such as proteins, lipids , DNA etc[2]. .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) by measuring serum Thiobarbituric acid reactive substances, vis-à-vis antioxidant status with estimation of plasma superoxide dismutase ,serum alpha-tocopherol. 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, it is one of the best studied metalloenzymes in human biochemistry. Alfa-tocopherol is chemically active antioxidant substance that acts as scavenger. It is well accepted fact that aging has a significant association with accumulation of free radicals and tocopherols are believed to delay the process of aging. 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.

MATERIAL AND METHODS:

Study population - 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.

STUDY DESIGN:

Hospital based noninterventional cross sectional study.

ETHICAL CLEARANCE AND INFORMED CONSENT: 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.

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**,** patient smoker, drinker, addicted to any substance**,** patient under any medication, chemotherapeutics or nutritional supplements

ANALYTICAL METODS

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[3] (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 Superoxide dismutase (SOD): Plasma SOD was measured by the method of Kakkar et al[4] (1989). 1.35ml of double distilled water, 50µl of plasma,1.2 ml of sodium pyrophosphate buffer (pH8.3),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 ºC 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

Assay of alfa-tocopherol: Serum tocopherol (vit E) was estimated by Baker & Flank's method [5](1968). Serum tocopherol was measured by their reduction of ferric to ferrous ion which then formed a colour complex with □-dipyridyl. Tocopherol being lipid soluble was first extracted in to xylene and reading of absorbance was taken at 460 nm against blank.

Statistical methods

Healthy human volunteers were selected according to pre set inclusion and exclusion criteria. Total 80 people 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 ± 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: 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: results are displayed in the form of Mean ± Standard deviation and Standard error of mean.

Parameters	Group A (Middle Aged) No of cases(n) = 40	Group B (Elderly) No of cases (n)=40
Serum TBARS (µmol/L) Mean±SD (SEM)	8.830 ± 0.466 (0.0783)	12.569 ± 0.490 (0.0775)
Superoxide dismutase(unit/ml) Mean±SD (SEM)	4.417 ± 0.430 (0.0681)	3.976 ± 0.475 (.0751)
alpha-Tocopherol (mg/L) Mean±SD (SEM)	8.457 ± 0.4372 (.06914)	6.457 ± 0.4635 (.07329)

Table 1 shows that Serum TBARS is significantly higher (12.57±0.490, p value <0.000) in elderly than middle aged population (8.83±0.467).Plasma SOD level is significantly lower (3.98±0.475, p value <0.000) in elderly than middle aged population (4.42±0.431).Serum alfa tocopherol is significantly lower (6.46±0.464, p value <0.000) in elderly than middle aged (8.46±0.437)

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 alpha-tocopherol	4.350	0.000	2.201	1.799
Super oxide dismutase	19.854	0.000	.6428	.2391

From table 2, It is evident that serum TBARS is significantly higher in elderly population than middle aged(p=0.000).Serum Superoxide dismutase and alpha -tocopherol activity are significantly lower in older age group.(p=.0.000).

Table 3: demonstrates 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**	**SOD**	**AlfaToco**
Age	1.00	**0.92**	**-0.39**	**-0.87**
TBARS	**0.92**	1.00	-0.40	-0.91
SOD	-0.39	-0.40	1.00	0.43
tocopherol	**-0.87**	-0.91	0.43	1.00

Correlation analysis shows There is significant positive correlation(r=0.92) between age and serum TBARS concentration. Plasma SOD negatively correlated (r=-0.39) with age. Significant negative correlation (r= -0.87) also exists between serum tocopherol concentration and age.

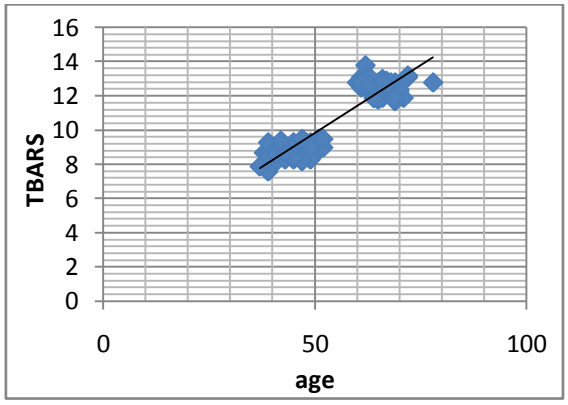

FIGURE 1: scatter chart showing correlation between age and TBARS

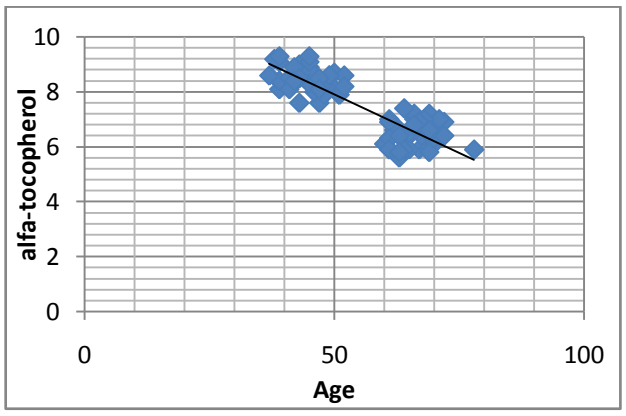

FIGURE 2: scatter chart showing correlation between age and alpha-tocopherol

DISCUSSION

Recently oxidative stress has captured considerable attention as a potential mechanism for aging process as well as various diseases. Harman[1] defines aging as the progressive accumulation of diverse changes in cells and tissues with advancing age that increase the risk of disease and death. Most of the theories claim that human body eventually succumb to overpowering force of damage caused by variety of environmental factors that are reactive with organic biomolecules. .The present study demonstrated that biomarkers of oxidative stress were greater in elderly subjects than middle aged subjects.

The present study included measurement of the concentration of TBARS for quantification of 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.. 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=o.ooo) in elderly population than middle aged population and there is a positive correlation (r=0.92) between age and TBARS concentration. Chan et al[6] in their Saopaulo 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 [7] & Block et al [8], 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 [9] in their work on European population postulated that TBARS production is dependent 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 [10] 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 Sl and Maurya Rk[11] observed a higher oxidative stress (increased MDA) in Indian population compared to values reported for European subjects. Saaswati M etal [12]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 urbanization and impact of different life style.

The principal micronutrient antioxidants are vitamin E, vitamin C and β-carotene. Some authors have stated that oxidative stress, aging & decline of vitamin E are interrelated. .It is observed in our study that α-tocopherol activity is significantly lower (p=0.0000) in elderly age group and α-Tocopherol has negative correlation (r=-0.87) with age. Mecocci et al[13]showed plasma levels of vitamin E in elderly is lower than younger in humans. The author concluded that vitamin E is of particular importance for longevity. Paolisso et al[14] also showed that plasma levels of vitamin E is lower in aged subjects than young adults. According to Junqueira[15], data from some literature show that plasma α-tocopherol appears to be increased with age .Again few studies found no effect of age in α-Tocopherol concentration[16] but Mino et al[17] noticed decrease in plasma α-Tocopherol concentration. Since fundamental mechanism of age related oxidative stress and role of antioxidants is not clearly understood, this subject still exists as a contentious issue among the scientists working on this subject matter.In addition, analytical differences between laboratories makes it difficult to compare the results obtained in different studies. In our endeavor 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. Dietary habits, lack of proper nourishment and necessary vitamin supplement etc may have some role in low concentration of vitamin E among elderly population. In view of increasing risk factors due to oxidative stress in human to various deadly diseases there has been a global trend towards the use of natural substances present in medicinal plants and dietary supplements as therapeutic antioxidant. Current research reveals that there is an inverse relationship between dietary intake of antioxidant rich food and incidence of human diseases [18]

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 etal [19] observed an age related decrease in Cu-Zn SOD activity. Guemouri et al [20] 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[21] 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.

In our endeavor 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.

Further investigation is needed encompassing larger sample size, other parameters of oxidative stress, dietary habit, genetic predisposition, per capita income and lifestyle of study population.

CONCLUSION

Our data suggests that oxidative stress, aging and decline in antioxidant status are not any disconnected phenomena; on the contrary, they are very much interrelated. Findings of the study highlight that free living elderly subjects are exposed to significant stress as we have observed that serum TBARs concentration which indicates greater lipid peroxidation is higher in elderly and antioxidants like plasma SOD and alpha tocopherol are lower in older subjects. Further investigation is needed encompassing other parameters of oxidative stress, dietary habits and genetic predisposition and life style of concerned population.

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