RESEARCH ARTICLE

Free Radicals and Ageing – Is There Any Correlation?

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ABSTRACT

Background: Ageing is a universal phenomenon for all the living subjects. Various theories exist for the process of ageing, but the highly accepted theory is "Free Radical Theory of Ageing" conceived by Harman. According to this theory there is some imbalance between production and scavenging mechanisms of free radicals. The free radicals produce damage over the lifetime which is critical in determining the life span of individual. In context of this theory the present study was conceived.

Objective: To investigate the correlation of free radicals and ageing process in human individuals.

Materials and Methods: Adult volunteers with the age of 21 to 80 yrs were divided into three groups of 21-40 yrs, 41-60 yrs and 61-80 yrs. In each group there were 30 individuals. The serum levels of Glutathione reductase (GSSG-reductase) and Total antioxidant status (TAS) were measured in all the individuals. The data was subjected to ANOVA. Then student's unpaired T-test was used for evaluation and the values of $P < 0.05$ were considered statistically significant. The data was also analyzed for correlation coefficient (r).

Results: The levels of GSSG-reductase and TAS were significantly lower in 61-80 yrs age group. There was negative correlation between ageing versus GSSG-reductase as well as TAS.

Conclusion: Oxidative stress is generated during various stages of life. The gradual but steady fall of the antioxidant GSSG-reductase and falling level of TAS provide reasonable indication of weakness in antioxidant shield for the individuals. It is concluded that there is correlation between generation of free radicals and ageing process.

KEY WORDS: Ageing; Free Radicals; Oxidative Stress; Glutathione Reductase; Total Antioxidant Status

INTRODUCTION

Ageing is an integral part of the process of growth and development that is terminated by death. It is sum of all the changes, which occur in man with the passage of time and leads to functional impairment of homeostasis ultimately leading to death. The aging process is a universal phenomenon.

Though phenomenal literature and huge theories of ageing are put forward, the most recent and highly accepted theory is "*Free Radical Theory of Ageing*" conceived by Harman.[1] In the free radical theory of aging, there is some imbalance between production and scavenging mechanisms of free radicals.[2] The free radical theory of aging proposes that reactive oxygen species (ROS) cause oxidative damage over the lifetime of the subject which is critical in determining the life span.[3] It is the cumulative and potentially increasing amount of accumulated damage that accounts for the dysfunctions and pathologies seen in normal aging.

Free radicals are now widely accepted as being very important, not only in the aging process but also in numerous human diseases/disorders where they have either a primary or secondary role. Currently, there are extensive global basic research efforts to define more clearly the role of free radicals and oxidative stress in these conditions. In context of this recent theory of ageing, this study was conceived. The objective of the study was to investigate the correlation between free radicals and ageing process.

MATERIALS AND METHODS

The levels of glutathione reductase (GSSG–reductase) and total antioxidant status (TAS) were studied in human individuals from different age groups. The approval from local ethical was obtained and written consent from all subjects was also taken.

Selection of Participants

Human individuals from different socioeconomic layers were randomly selected. They were not suffering from any major illness like diabetes, tuberculosis, cancer etc. at the time of study as well as in the past. They were divided into the three different age groups of 21-40 yrs, 41-60 yrs and 61-80 yrs. In each group 30 subjects were included and blood samples were collected in plain bulbs. After clot formation the samples were centrifuged at 2000 rpm for 5 minutes and serum was separated. GSSG-reductase and TAS levels were estimated in serum. The kits were purchased from Randox Laboratory Limited, Diamond road, Crumlin, Co. Antrim, U.K. The analysis was done on spectrophotometer in Immune laboratory, Govt. Medical College, Surat.

Glutathione Reductase

GSSG-reductase catalyzes the reduction of oxidized glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADP^+$. The decrease in the absorbance is measured at 340 nm. This method was described by Goldberg and Spooner.[4] Assay Principle:

$NADPH + H^+ + GSSG \Rightarrow NADP^+ + 2GSH$

Total Antioxidant Status

TAS was measured as described in the method of Miller et al.[5] ABTS (2,2' – Azino – di - [3 - ethilbenzathiazolin sulphonate]) was incubated with peroxidase (metmyoglobin) and H_2O_2. This leads to the production of the radical cation $ABTS^+$ with a relatively stable blue-green color, which was measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree, which is directly proportional to their concentration. Assay Principle:

$HX\text{-}Fe^{III} + H_2O_2 \Rightarrow X - [Fe^{IV} = O] + H_2O$

$ABTS + X - [Fe^{IV} = O] \Rightarrow ABTS^+ + HX\text{-}Fe^{III}$

$HX\text{-}Fe^{III}$: Metmyoglobin; $X - [Fe^{IV} = O]$: Ferrylmyoglobin; ABTS: 2,2' – Azino – di - [3 - ethilbenzathiazolin sulphonate]

Statistical Analysis

The data was subjected to ANOVA. Then student's unpaired T-test was used for evaluation and the values of $P < 0.05$ were considered statistically significant. The data was also analyzed for correlation coefficient with using open EPI software.

RESULTS

Glutathione Reductase

The mean value of GSSG-reductase for age group of 21-40 yrs is 63.66 ± 2.5 U/l and for group of 41-60 yrs is 57.97 ± 2.64 U/l which is 8.9 % less but not significantly different. Mean value for age group of 61-80 yrs is 45.68 ± 2.4 U/l, which is 21 % less as compared to second group. This is statistically highly significant from both the age group of young and middle one. The value of correlation coefficient (r) between age and GSSG-reductase is -0.4 (Table-1).

Total Antioxidant Status

The mean value of TAS for age group of 21-40 yrs was 1.56 ± 0.02 mmol/l and for group of 41-60 yrs was 1.47 ± 0.02 mmol/l which is 5.8 % less but not statistically significant. Mean value of TAS for age group of 61-80 yrs was 1.24 ± 0.03 mmol/l, which was 15.6 % less than second group. This is statistically significant. The value of correlation coefficient (r) between age and total antioxidant status is -0.7. (Table-2)

Table-1: GSSG-Reductase (U/l) in Different Age Groups

	A	B	C	F statistics	P value
Age Group	21 - 40	41 - 60	61 – 80	13.25	0.000
No. of subjects	30	30	30		
Mean± SD	63.66 ± 13.73	57.97 ± 14.50	45.68 ± 13.21*,#		

* P < 0.01 for group B and group C
#, P < 0.01 for group A and group C

Table-2: Total Antioxidant Status (mmol/l) in Different Age Groups

	A	B	C	F statistics	P value
Age Group	21 - 40	41 - 60	61 – 80	52.14	0.000
No. of subjects	30	30	30		
Mean± SD	1.56 ± 0.02	1.47 ± 0.02	1.24 ± 0.03*		

* P < 0.05 for group C and group A

DISCUSSION

Ageing is a universal phenomenon that is obvious and inevitable, yet poorly understood. We, humans are constantly exposed, throughout life, to a wide variety of extrinsic and intrinsic agents, which have the potential to damage cellular biomolecules, including DNA. Many theories have been proposed to explain the ageing process, but recently free radical theory of ageing has been drawing attention and thereby opening the doors for possible utilization of antioxidants for prevention or even reversal of the ageing process.

Recently increasing interest is seen in the role of free radicals and oxidative damage in a variety of pathophysiological processes. Free radicals are chemical species having an unpaired electron. Free radicals are considered as fragments of molecules and generally very reactive.[6]

The free radicals like hydroxyl radical, superoxide anion, peroxyl radicals and hydrogen peroxide are formed in different metabolic processes and generate oxidative stress. Production of these radicals increases oxidative stress and in the presence of ions of metals, leads to the creation of more reactive metabolites.[7] The origin, reactivity with other biomolecules and removal of free radicals, is in the foreground of interest since their effects are mostly toxic and result in a whole series of pathological changes in cells, organs and whole organisms.

Free radicals can cause extensive damage to DNA, protein, and lipid.[8] Free radicals are scavenged by the antioxidants, which are the main line of defense to protect our body from their deleterious effects. All the endogenous antioxidants (glutathione enzymes, urate, ceruloplasmin etc.) and exogenously administered antioxidants (acorbate, carotenoids, alpha tocopherol etc.) comprise total antioxidant status of any individual. The balance between the increased generation of free radicals in various pathological states, or unfavorable conditions in the environment, and decreased natural antioxidants (vitamin C, E, glutathione etc.) or enzyme character (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase etc.), plays the chief role in damage, which is the cause of many diseases and ageing.

In our study correlation coefficient for age and GSSG-reductase (r = –0.4) indicates a poor negative correlation of age with GSSG-reductase.

Similarly in a study carried out by Ceballos-Picot et al[9] a negative correlation was found between age and activities of SOD, GSSG-reductase and glutathione-S-Transferase. Matsubara and Machado[10] when examined erythrocyte glutathione system in different age groups of healthy people, also obtained similar results. Total glutathione, GSSG-reductase and glutathione peroxidase levels were significantly higher in the young group than in the elderly group. In the present study the value of GSSG-reductase indicate similar results.

Total antioxidant status gives a clue about individual's ability to stand against oxidative stress. To elucidate the possible involvement of free radicals in ageing process we have also measured TAS level in different age groups. Results of the present study show significant reduction in total antioxidant status of aged (61-80 yrs) healthy individuals in comparison with younger (21-40 yrs) individuals. Correlation coefficient for TAS and age (r = -0.7) indicates a strong negative correlation; i.e. as age increases TAS level decreases.

In one of the study related to aging by Benot et al[11], similar findings were obtained. They found that nocturnal serum values of TAS exhibited maximal values during the first four decades; thereafter, these values decreased as age advanced.

Compelling evidences indicate that free radicals are important mediators of ageing process. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical. The mitochondrial DNA is susceptible to oxidative damage by the increasing levels of free radicals in the mitochondrial matrix, leading to erosion of antioxidant shield. Spilling over of free radicals into the cytoplasm can further aggravate damage to the various microorganelles. Radicals formed in this way can activate proteolytic enzymes (proteinases), which can cause break down of collagen and other components of the extracellular matrix leading to degenerative process. Many reports are indicative of excessive free radical generation with age-related disorders like atherosclerosis, cancer, cataract, dementia, Parkinson's disease, and Alzheimer disease.[12-14]

CONCLUSION

In conclusion ageing process shows positive relationship with free radical generation and thereby with oxidative stress. All the antioxidants fades away in the senile age group, thus remove the protective shield of an individual. Enhanced generation of free radicals in the later part of the life especially after 60 yrs of age, snatches away the vitality of most individuals, ultimately leads to the bitter truth of life, which is DEATH.

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