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Alterations in Lipid Peroxidation and Certain Antioxidant Enzymes in Different Age Groups under Physiological Conditions

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ABSTRACT Free radicals and peroxides are involved in physiological phenomena such as synthesis of prostaglandin and thromboxanes and are thought to participate in aging process. Free radicals are also responsible for the pathogenesis of various diseases like atherosclerosis, inflammatory diseases and malignancies etc. The objective of the present work was to investigate the possible role of free radicals in aging process by studying age related changes in lipid peroxidation and in certain antioxidant enzymes like xanthine oxidase, superoxide dismutase, glutathione peroxidase and glutathione. The level of lipid peroxidation and antioxidant initiating enzyme xanthine oxidase and antioxidant scavenging enzymes like superoxide dismutase, glutathione peroxidase and glutathione was estimated in 300 healthy male and females ranging in age from 15 to 65 years. The level of xanthine oxidase enzyme and lipid peroxidation was significantly increased while the levels of antioxidant defense enzymes such as superoxide dismutase and glutathione peroxidase and glutathione were deceased significantly in plasma with the increase of age. Our observations are suggesting that old age is associated with an increase in systematic oxidative stress.

INTRODUCTION

Aging is the time related deterioration of the physiological functions, leading to the cell's inability to withstand external and internal stress. The aging process is slow in the early stages of life but rapidly increases in later stages due to the exponential nature of the process. The causative factors for the time dependant deleterious process of aging are yet not well defined and no single adequate molecular explanation for aging is currently available (Harman 1992, Timiras 1994). The free radical theory of aging has received widespread attention **(**Harman 1988, Kasapoglu and Ozben 2001**).** Free radicals are generated by redox reactions that occur during normal physiological processes and also contributed by environmental sources, cause cell damage by autocatalytic lipid peroxidation of membranes, lesions in DNA, and cross-linkage in proteins. This deleterious action of free radicals is thought to be responsible for the functional deterioration associated with aging (Timiras 1994 and Afanas 2005).

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The antioxidant system, the first line of defense against free radicals includes a number of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and non-enzymatic antioxidants such as glutathione (GSH), protein-SH, ascorbic acid and dietary antioxidants etc. According to the free radical theory of aging, one might expect the activity of antioxidant enzymes to be altered with increasing age **(**Harman 1988, Afanas 2005 and Rizvi and Maurya 2007). This free radical mediated peroxidative injury has a role in pathological changes of aging. So, in the present study, we evaluated the level of serum lipid peroxidation (LPO) and antioxidant initiating enzyme such as xanthine oxidase (XOD) and antioxidant defense enzymes like SOD, GPx and GSH) to observe the alteration in oxidant: antioxidant system in healthy human subjects with increasing age.

MATERIAL AND METHODS

Subjects: The present study was conducted on 300 healthy subjects of both sexes in the age group ranging from 15 to 65 years (Table 1). All subjects were submitted to through clinical examination to detect signs or symptoms of chronic disease such as arterial hypertension, heart failure, diabetes mellitus or chronic anemia,

Table 1: Distribution of subjects into different groups on the basis of age and sex

Groups	*Age (in years)*	*Males*	*Fe-males*	*Total [Males +Females]*
Group-1	15 – 30	50	50	100
Group-2	31 – 45	50	50	100
Group-3	46 – 65	50	50	100
Total	15 - 65	150	150	300

Individuals with chronic disease and/or under drug treatment were excluded from the study. None of the subject was a cigarette smoker/ alcoholic. These subjects were divided into three different groups. Group-1: 15 - 30 years (23.88 ± 4.63), Group-2: 31 - 45 years (36.60 ± 3.71), Group-3: 46 - 65 years (51.36 ± 4.16). 100 subjects (Male and Female) were recruited in each group (Table 1 and 2).The body weight of the subjects in all the three groups ranged form 54.28 ± 7.50 Kg to 71.96 ± 6.42Kg (Table 3). In case of females, only non pregnant females included in the present study.

Sample Collection: 10ml of blood was drawn after over night fasting with a dry disposable syringe and needle, under all aseptic conditions by venepuncture in the anticubital vein in sterile, dry and acid washed vials. 5ml of blood was collected in the plain vial for the separation of serum which was used for estimation of Lipid Peroxidation levels and 5ml of blood was collected in ethylenediaminotetra acetic acid (EDTA) for the separation of plasma and this plasma was used for the estimation of xanthine oxidase, superoxide dismutase, glutathione and glutathione peroxidase levels. All the biochemical assays were analyzed immediately after blood collection from individuals.

Biochemical Assays

1. Xanthine oxidase (XOD): The activity XOD was measured by the method of Fried and Fried (1974) using nitro blue tetrazolium (NBT) which formed farmazan. The increase in the intensity of color with time was measured spectrophotometrically at 540 nm for 10 minutes.

2. Lipid Peroxidation(LPO): LPO level in serum was estimated by measuring the pink colored chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Satoh (1978).

3. Superoxide dismutase (SOD): The activity of SOD was assayed by applying the method of Kono (1978). The activity of SOD was measured by monitoring the rate of inhibition of NBT reduction. One unit is defined as the amount of enzyme, which caused half-maximal inhibition of NBT reduction.

4. Glutathione (GSH): GSH level in plasma was estimated by the method of Beutler et al. (1963), using 5-5' dithiobis2-notrobenzoic acid (DTNB).

5. Glutathione peroxidase (GPx): GPx activity in plasma was measured by using hydrogen peroxide as a substrate by applying the method of Rotruck et al. (1973).

Statistical Analysis: Numerical data was presented as mean values ± S. D, was calculated separately for all the groups. The statistical significance was evaluated by student's 't' test and $p \leq 0.05$ was considered statistically significant.

RESULT AND DISCUSSION

The activity of XOD, a superoxide initiating enzyme was found to be significantly increased ($p < 0.001$, from 2.96 ± 0.005 to 3.49 ± 0.007 mg/ml) with increase in age group (Table 4 and 5). XOD, a highly versatile enzyme i.e. widely distributed among species from bacteria to human. XOD exists predominately as NAD^+ dependent xanthine dehydrogenase (XDH) that itself has no role in

Table 2: Age of subjects in years in each group (Mean ± SD).

Groups	*Males (in years)*	*Females (in years)*	*Mean [Males + Females] (in years)*
Group-1 (15 – 30)	25.40 ± 4.29	22.36 ± 4.54	23.88 ± 4.63
Group-2 (31 – 45)	39.60 ± 3.40	36.60 ± 3.71	38.10 ± 3.83
Group-3 (46 – 65)	54.04 ± 4.31	51.36 ± 4.16	52.70 ± 4.40

Table 3: Body weight (Kg) of subjects of different groups (Mean ± S. D.).

Groups	*Males(n=50)*	*Females(n=50)*	*Mean [Males + Females](n=100)*
Group-1(15 - 30)	58.68 ± 13.50	54.28 ± 7.50	56.48 ± 10.5
Group-2(31 - 45)	62.92 ± 5.18	64.88 ± 6.73	63.90 ± 6.13
Group-3(46 - 65)	71.84 ± 5.88	71.96 ± 6.42	71.90 ± 6.01

Table 4: The effect of aging on XOD, LPO and SOD levels in normal healthy subjects (Mean ± S. D.).

Groups	*XOD(U/ml)*	*LPO(n mol/ml)*	*SOD(U/ml)*
Group-1(15 - 30)	2.976 ± 0.05	2.735 ± 0.60	2.615 ± 0.043
Group-2(31- 45)	3.14 ± 0.08(+6.08)	3.450 ± 0.33(+30.68)***	2.130 ± 0.055(-16.78)**
Group-3(46 - 65)	3.49 ± 0.07(+ 17.90)**	4.380 ± 0.69(+65.91)***	1.885 ± 0.036(-33.21)***

Values in parentheses represent percentage change with respect to group-1
**p≤0.01, ***p≤0.001

Table 5: The effect of aging on XOD, LPO and SOD levels in normal healthy males and females subjects. (Mean ± S. D.).

Groups	*XOD (mg/ml)*	*LPO (n mol/ml)*	*SOD (U/ml)*
Group-1: (15 - 30)			
Male (n=50)	1.49 ± 0.009	2.85 ± 0.064	2.57 ±0. 042
Female (n=50)	1.47 ± 0.010 (-1.34) NS	2.62 ± 0.031 (-8.07) NS	2.66 ± 0.036 (+3.50) NS
Group-2: (31- 45)			
Male (n=50)	1.56 ± 0.011	3.61 ± 0.027	2.12 ± 0.050
Female (n=50)	1.58 ± 0.015 (+1.28) NS	3.29 ± 0.031 (-8.86) NS	2.14 ± 0.056 (+0.943) NS
Group-3: (46 - 65)			
Male (n=50)	1.70 ± 0.009	4.54 ± 0.072	1.95 ± 0.028
Female (n=50)	1.79± 0.013 (+5.29) NS	4.22 ± 0.064 (-7.04) NS	1.82 ± 0.039 (-6.66)NS

Values in parentheses represent percentage change with respect to females.
NS: Not Significant.

the initiation or potentiation of oxidative damage in cells. However, in many pathological conditions XDH is converted into XOD (Granard et al. 1981 and Xu et al. 1984). The conversion of XDH into XOD is suggested to occur in damaged cells (DeGroot and Littauer 1988 and Batteli et al. 1992). XOD catalyses the oxidation of hypoxanthine/ xanthine to uric acid and generates superoxide radicals ($O_2^{\cdot-}$). Hydrogen peroxide formed from $O_2^{\cdot---}$ could be converted into highly reactive hydroxyl radical ($HO^{\cdot}$) leading to oxidative stress as a result of oxidation of biological molecules. In the present study, a significant increase ($p<0.001$) in XOD level with increase of age may produce a burst of free radicals.

Once superoxide radical is produced then hydrogen peroxide and hydroxyl radical are continuously produced by a Haber-Weiss reaction and /or Fenton type reaction (Gill et al. 2002 and Nonaka et al. 1989) Oxygen radicals might cause the lipid peroxidation of biomembrane through a chain reaction. The first step is the initiation reaction, which begins by taking out "H" in unsaturated fatty acid by oxygen radicals. The second is the propagation and the final step is termination. The extent of lipid peroxidation has often been determined by the thiobarbituratic acid (TBA) test, which has also been considered for the detection of malondialdehyde. A significant increase ($p<0.001$) from 2.735 ± 0.60 to 4.380 ± 0.69 n mol/ml in malondialdehyde (representing the lipid peroxidation) level with increase in age group (Table 4 and 5) was observed in the present study, might lead to susceptibility of the biomembrane, which ultimately leads to tissue injury/ damage.

SOD, a superoxide radical scavenging enzyme level was found to be significantly decreased from 2.615 ± 0.43 to 1.885 ± 0.036 U/ml with increase in age group (Table 4 and 5). SOD is a first line of defense against the superoxide radical. The amount of SOD is organ specific. Three types of SOD have been purified; CuZn-SOD, Mn-SOD (McCord 1979) and extracellular – SOD (EC-SOD) (Marklund, 1984). CuZn-SOD is found abundance in cytosol. CuZn- SOD consists two protein subunits each has an active site containing one Cu ion and one Zn ion (McCord 1979). Cu ion serves as active redox site and Zn ions maintain the protein structure (Frivich, 1975). Mn-SOD is located in mitochondrial matrix (Bast and Haenen 1984). It has four subunits with Mn in each subunit. EC-SOD is present in plasma, bound to heparin sulfate on the surface of endothelial cells. EC-SOD is tetrameric glycoprotein, which contains Cu and Zn ion. The presence of SOD in various compartments of our body enables it to dismutate superoxide radicals immediately and protects the cells from oxidative damage. Attenuation in the

activity of SOD was also reported by several other authors in different species with aging (Frivich 1975; McCord 1979; Bast and Haenen 1984 and Marklund 1984**).** The decrease in activity of SOD with increasing age group was also observed in the present study, may reflect the tissue injury due to accumulation of O^{-}_{2} radicals.

Glutathione (GSH) a tripeptide is maintained in reduced state by an efficient glutathione peroxidase/glutathione reductase system. Glutathione, a potent endogenous antioxidant that helps to protect cells from a number of noxious stimuli including oxygen derived free radicals (Bast and Haenen, 1984). A significant decrease in the level of glutathione might be accompanied by a significant increase in MDA level. In the present work, the level of glutathione significantly decreased from 38.65 ± 2.60 to 29.79 ± 1.69 n mol/ml in both sexes with increase of age (Table 6 and 7). This observation is in agreement with the reports that inverse relationship exists between lipid peroxidation and glutathione status (Curello et al. 1985; Hill and Singal 1996; Singal et al. 1993 and Blum and Fridovich 1985). Glutathione depletion of 20% to 30% can impair the cell defense against the toxic action of xenobiotic and may lead to cell injury/death (Mezzetti et al. 1990 and Mimic et al. 1995).

The activity of GPx, a selenium-containing enzyme was found to be decreased from 6.64% to 20.79% with respect to group 1 (Table 6 and 7). GPx catalyses the reduction of variety of hydrogen peroxide (ROOH and H_2O_2) using glutathione as a substrate, thereby protecting mammalian cells against oxidative stress (Blum and Fridovich 1985 and Nakazawa et al. 1996). It is well reported that low activity of this enzyme may render the tissue more susceptible to lipid peroxidation damage (Netelson 1957 and Raes et al. 1987). Accordingly,

Table 6: The effect of aging on GSH and GPx levels in normal healthy subjects (Mean ± S. D.).

Groups	*GSH(n mol/ml)*	*GPx(U/ml)*
Group-1(15 - 30)	38.65 ± 2.60	0.527 ± 0.003
Group-2(31- 45)	34.56± 2.30 (-10.58)*	0.492 ± 0.004 (-6.64)
Group-3(46 - 65)	29.79 ± 1.69 (-22.92)***	0.417 ± 0.002 (-20.79)**

Values in parentheses represent percentage change with respect to group-1
**pd"0.01, ***pd"0.001

Table 7: The effect of aging on GSH and GPx levels in normal healthy males and females subjects (Mean ± S. D.).

Groups	*GSH (n mol/ml)*	*GPx (U/ml)*
Group-1: (15 - 30)		
Male (n=50)	19.66 ± 0.64	0.521 ± 0.003
Female (n=50)	18.99± 0.31 (-3.41)^{NS}	0.533 ± 0.004 (+2.30) ^{NS}
Group-2: (31- 45)		
Male (n=50)	17.62 ± 0.27	0.485 ± 0.004
Female (n=50)	16.94 ± 0.31 (-3.86) ^{NS}	0.499 ± 0.004 (+2.88) ^{NS}
Group-3: (46 - 65)		
Male (n=50)	15.26 ± 0.72	0.410 ± 0.002
Female (n=50)	14.50 ± 0.64 (-4.78) ^{NS}	0.425 ± 0.003 (+3.65) ^{NS}

Values in parentheses represent percentage change with respect to females.
NS: Not Significant

in the present work, we observed a significant decrease ($p<0.01$) in GPx activity from 0.527 ± 0.003 U/ml to 0.417 ± 0.002 U/ml (Table 6 and 7)) in plasma with increasing age groups in both sexes upon increase in LPO level (Table 4 and 5). This observation is in accordance with the hypothesis that LPO and GPx might play a role in tissue damage (Raes et al. 1987; Mezzetti et al. 1990; Lang et al. 1992 and Mimic et al. 1995). No Significant change was observed in the levels of LPO, XOD, SOD, GSH and GPx on comparison between male and female subjects with the same age group (Table 5 and 7). Thus sex of the subjects has no effect on the levels of LPO and antioxidant enzymes with the increase of age group.

In conclusion, the aforementioned observations are suggesting that increasing age might be responsible for the induction of oxidative stress in healthy subjects by altering the levels of LPO, XOD, SOD, GPx and GSH and to counter these changes dietary supplementation of a variety of antioxidants might be beneficial.

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