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Kaur S
Aggarwal R
Gupta K

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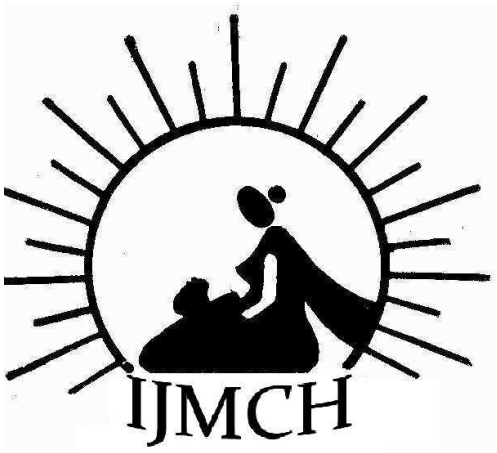

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To determine levels of lipid peroxidation and antioxidant activity and its relation with aging in females of Bathinda district of Punjab.

A Study of Lipid Peroxidation and Antioxidant Activity and its Relation with Aging in Females of Bathinda District of Punjab

Kaur S,*^ Aggarwal R,*¥ Gupta K*+

***Assistant Professor**

^Department of Physiology, ¥Department of Community Medicine, +Department of Biochemistry

Adesh Institute of Medical Sciences and Research, Bathinda

Correspondence: Dr. Sandeep Kaur

Email: sandeepkular72@yahoomail.co.in

ABSTRACT

Research Question: To determine levels of lipid peroxidation and antioxidant activity and its relation with aging in females of Bathinda district of Punjab.

Settings and design: Hospital based study

Participants: 300 females of age 15 – 65 years.

Methodology: Subjects selected were healthy female attendants of patients visiting various OPDs at Adesh Institute of Medical Sciences and Research, Bathinda. A pretested performa was used to study each subject. Malondialdehyde, the end product of lipid peroxidation reaction, was measured to assess the level of oxidative stress that is produced as a result of damage to cell membrane lipids by free radicals. Antioxidants superoxide dismutase (SOD), glutathione peroxidase (GPx) levels were estimated to determine the extent of protection against these oxidants. Statistical analysis was carried out by Student's t-test. The data was expressed as mean ± SD and the p value≤ 0.05 was considered significant.

Results: The levels of lipid peroxidation in females increased while the levels of antioxidant defense enzymes such as superoxide dismutase and glutathione peroxidase decreased significantly with increase in age.

Key Words: ***Aging, Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx)***

INTRODUCTION

Aging is the time related deterioration of the physiological functions, leading to the cell's inability to withstand external and internal stress. [1] Free radicals are generated by redox reactions that occur during normal physiological processes and also contributed by environmental sources. All the major classes of biomolecules may be attacked by free radicals but the lipids are probably the most susceptible. [2] Cell membranes are rich sources of polyunsaturated fatty acids (PUFAs) which are readily attacked by oxidizing radicals. The oxidative destruction of PUFAs, known as lipid peroxidation is particularly damaging because it precedes a self perpetuating chain reaction. [3] Malondialdehyde (MDA) is the major reactive aldehyde resulting from peroxidation of biological membrane. [4] Detoxification of reactive oxygen species is one of the prerequisites of aerobic life, and many defenses have evolved providing an important antioxidant defense system of prevention, interception and repair. These defenses consist of nonenzymatic scavengers and quenchers as well as enzymatic systems including superoxide dismutases(SOD) and hydroxyperoxidases such as catalase and glutathione peroxidase.[5] The excessive production of free radicals in the organism and the imbalance between the concentrations of free radical & antioxidant defenses is thought to be related to various patho-physiological conditions like aging.[6] Considering the co-morbidities like atherosclerosis, inflammatory diseases and malignancies associated with aging and its implications on health, this study aims to determine the variation in levels of lipid peroxidation and antioxidant activity in females so that lifestyle and dietary modifications may be advised with increase in age.

MATERIALS AND METHOD:

Subjects selected were female attendants of patients visiting various OPDs at Adesh Institute of Medical Sciences and Research, Bathinda. The study was conducted in the Department of Physiology, Adesh Institute of Medical Sciences and Research, Bathinda, on 300 subjects in the age group ranging from 15 to 65 years, with approval of Ethics committee. All females were submitted to detailed clinical examination to detect signs or symptoms of any chronic disease such as arterial hypertension, heart failure, diabetes mellitus or chronic anemia. Subjects found pregnant or affected by a chronic disease or taking any drug treatment/vitamins and mineral supplements were excluded from this study. Informed consent was taken from all the subjects. Subjects were categorized into three age groups: Group I: 15-30 years; Group II: 31-45 years and Group III: 46-65years.

Age and weight was recorded. Weight was taken on a weighing scale with standard minimum clothing to the nearest 0.5kg

Sample Collection: 10ml of blood was drawn after over night fasting under all aseptic conditions. 5ml of blood was collected in the plain vial for the separation of serum which was used for estimation of lipid peroxidation (malondialdehyde) and superoxide dismutase levels. Malondialdehyde (MDA) was estimated in terms of thiobarbituric acid reactive species (TBARS) by the method of Satoh.[7] Superoxide Dismutase (SOD) activity in serum was assayed by using the method of Marklund and Marklund.[8] 5ml of blood was collected in vial containing ethylenediaminotetraacetic acid (EDTA) for the separation of plasma and

this plasma was used for the estimation of glutathione peroxidase levels by using hydrogen peroxide as a substrate by applying the method of Rotruck et al.[9]

Statistical Analysis Numerical data presented as mean ± SD, was calculated separately for all the groups. The statistical significance was evaluated by student's t test and p≤ 0.05 was considered statistically significant.

OBSERVATIONS

The following observations were made:

Table I: Distribution of Female subjects according to Age and Weight

Age -groups(years)	No of subjects	Mean ± SD(years)	Mean weight(kg)
I (15-30)	100	22.36 ±4.54	54.28 ± 7.50
II (31-45)	100	36.60 ± 3.71	64.88 ± 6.73
III (46-65)	100	54.04 ± 4.31	71.96 ± 6.42

Table I depicts average age in years of females in Group I Group II Group III was 22.36 ±4.54, 36.60 ± 3.71 and 54.04 ± 4.31 respectively. The average body weight of the subjects in Groups III was more than in Group I and II.

Table II a: Serum lipid peroxidation levels (nmol/ml) in healthy females of different age groups

Age Group (yrs)	Serum lipid peroxidation (nmol/ml)
	Mean ± SD
I (15-30)	2.64 ± 0.64
II (31-45)	3.45 ± 0.33
III (46-65)	4.38 ± 0.69

Table IIa depicts that the levels of malondialdehyde (representing the lipid peroxidation) showed a linear increase with increase in each age group. Group III (46-65years) subjects had higher malondialdehyde levels than Group I and Group II subjects.

Table II b depicts a highly significant percentage change in lipid peroxidation levels of group II and group III as compared to Group I females. Significant increase in levels of lipid peroxidation was also seen in Group III females as compared to Group II females.

Table II b: Comparison in levels of lipid peroxidation in healthy females of different age groups

Group	Percent change	't' value	p value	Significance
I Vs II	30.68	t=7.99;	p<0.001	Highly Significant
I Vs III	65.91	t=13.14;	p<0.001	Highly Significant
II Vs III	26.96	t=8.64;	p<0.001	Highly Significant

Table III a: The effect of aging on serum Superoxide Dismutase (SOD) levels in healthy females subjects

Age Group (yrs)	SOD (units/ml)
	Mean ± SD
I (15-30)	2.92 ± 0.36
II (31-45)	2.44 ± 0.56
III (46-65)	1.72 ± 0.39

Table III a depicts that levels of SOD, a superoxide radical scavenging enzyme, had an inverse relationship with increase in age. Levels of serum SOD were least in Group III and maximum in females of Group I**.**

Table III b: Comparison of Serum Superoxide Dismutase levels in Females of different Age groups

Group	Percent change	't' value	p value	Significance
I Vs II	16.44	t =3.64;	P <0.001	Highly Significant
I Vs III	41.10	t=11.42;	P <0.001	Highly Significant
II Vs III	29.50	t =5.33;	P <0.001	Highly Significant

Table III b depicts highly significant percentage decrease in level of superoxide dismutase in Gp-II (31-45yrs) and Gp-III (46-60 yrs) subjects when compared with Gp-I (15-30yrs) subjects.

Table IV a: Effect of Aging on Glutathione Peroxidase (GPx) levels in Healthy Female subjects

Age Group (yrs)	GPx (U/ml)
	Mean ± SD
I (15-30)	0.523 ± 0.07
II (31-45)	0.47 ± 0.11
III (46-65)	0.412 ± 0.08

Table IV a depicts that the level of glutathione peroxidase decreased significantly from 0.523 ± 0.07 to 0.412 ± 0.08 U/ ml in females with increase of age

Table IV b : Comparison of Glutathione Peroxidase *(*GPx) levels in Females of different Age groups

Group	Percent change	't' value	p value	Significance
I Vs II	10.1	t =2.03	< 0.05	Significant
I Vs III	21.2	t=5.22	<0.001	Highly Significant
II Vs III	12.3	t =2.13	< 0.05	Significant

Table IV b depicts highly significant percentage change in level of glutathione peroxidase in Group III (46-65yrs) with respect to Group I (15-30yrs).

DISCUSSION

The present study conducted on females of Bathinda district of Punjab, observed a significant increase ($p<0.001$) from 2.735 ± 0.60 to 4.22 ± 0.64 n mol/ml in malondialdehyde (representing the lipid peroxidation) levels with increase in age. The observed increase in lipid peroxidation is consistent with other studies although variation in levels may be seen from region to region. Plasma lipid peroxide levels in Nauruan population were found to be 5.78 ±3.21 nmol/ml in 35-44 years age group as compared to 4.03 ± 1.94 nmol/ml in 20-24 years age subjects.[10] Similarly increased levels of malondialdehyde/ml were observed in 51-60 years old subjects as compared to 11-20 years subjects in population around Ambajagoi.[11] Levels of lipid peroxides, also showed positive co-relation with age in a study conducted in Tirupati, in both rural and urban Indian men less than 70 years.[12] Activation of free radicals oxidation processes and decrease in antioxidant defense activity was found in the group of older and old subjects (60-80 years) as compared to younger subjects (20-29 years).[13] This increased free radical mediated lipid peroxidation with age in different populations may be due to change in redox potential of the cell with increase in age.[14]

In the present study levels of SOD, a superoxide radical scavenging enzyme were found to be significantly decreased from 2.92 ± 0.36 to 1.72 ± 0.39 U/ml with increase in age group. Also levels of Glutathione peroxidase, a selenoenzyme which catalyzes the reduction of hydroperoxides at the expense of reduced glutathione, reduced from 0.523 ± 0.07 to 0.412 ± 0.08 U/ ml with increase in age. Similar findings have been observed in other studies. European men also suggested a progressive and slow decline of antioxidant status in healthy free-living elderly subjects.[15] Age dependant decrease in antioxidant capacity of tissues reflected by decreased plasma GSH levels was observed in sportsperson which were partly compensated by physical training.[16] Even erythrocyte GSH-Px activity was significantly less in the elderly than in the young group.[17] Moreover decrease in SOD activity in the present study was seen in subjects less than 65 years when the oxidative stress might not be increased sufficient enough to up regulate the enzyme as was observed in elderly subjects in some studies .[18, 19, 20]

Our findings of decrease in SOD activity with age were in the serum which may be different from that of tissues. SOD activity determined in the skeletal muscle was found to be lower but Glutathione peroxidase (GPx) activity remained unchanged in the 66-91 year-old subjects as compared to 17-40 year-old subjects.[21]

The results of this study indicate that there is decrease in antioxidant activity and increase in lipid peroxidation with age. Alteration in levels of lipid peroxidation and antioxidant enzyme activity seen with advancing age may explain probable cause of aging and age-related disease in females.

CONCLUSION

In the present study, increase in lipid peroxidation and decrease in antioxidant enzyme activity, measured by SOD and glutathione peroxidase was observed with increase in age. Increase in lipid peroxidation suggests role of free radicals in various pathophysiological changes observed with increase in age. Awareness must be generated in females that increase in age is associated with decrease in antioxidant activity so dietary supplementation by a variety of antioxidants must be taken. Although calorie and fat restriction may be advised to females as age advances but intake of green vegetables, fruit and nuts rich in antioxidants must be emphasized for a long, healthy and independent life.

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