

Decrease in antioxidant status of plasma and erythrocytes from geriatric population

Manjulata Kumawat^{a,1}, Tarun Kumar Sharma^{a,1,*}, Ishwar Singh^{b,1}, Neelima Singh^{c,1},
Sanjeev Kumar Singh^{c,1}, Veena Singh Ghalaut^{a,1}, Vijay Shankar^a and Satish Kumar Vardey^{d,1}

^aDepartment of Biochemistry, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak, Haryana, India

^bDepartment of Neurosurgery, Pt. B.D. Sharma University of Health Sciences, PGIMS, Rohtak, Haryana, India

^cDepartment of Biochemistry, G.R. Medical College, Gwalior, Madhya Pradesh, India

^dDepartment of Biochemistry, S.M.S. Medical College, Jaipur (Rajasthan), India

Abstract. *Background:* Ageing is associated with an accumulation of free radical damage, which leads to physiological and clinical modifications. The study aims to find out the status of lipid profile, antioxidant enzymes, malondialdehyde in geriatric population.

Patients/methods: The study was conducted on 150 subjects (75 healthy control between the ages of 20–30 years and 75 elderly subjects between ages of 50–70 years as cases). The following parameters were analyzed using the standard reference methods: lipid profile, reduced glutathione, glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase and malondialdehyde.

Results: The present study was conducted to estimate the oxidative stress parameters in geriatric population. Highly significant increase in total cholesterol (TC), triglyceride (TG), LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C), malondialdehyde, catalase and decrease in high density lipoprotein cholesterol (HDL-C), reduced glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase was observed in geriatrics when compared with their younger counterparts.

Conclusion: This study concluded that there is enhanced oxidative stress and decreased antioxidant defence in geriatrics as compared to younger subjects which could play an important role in ageing. Dyslipidemia has become one of the important risk factors for the increasing prevalence of cardiovascular diseases. There is lack of awareness on the relationship between blood lipids and the risk of cardiovascular diseases in geriatric population. The strategy of early prevention should be adopted against dyslipidemia.

Keywords: Oxidative stress, malondialdehyde, antioxidants, geriatric population

List of abbreviations

CAT Catalase;
MDA Malondialdehyde;
GSH Reduced glutathione;
GR Glutathione reductase;
GPx Glutathione peroxidase;

SOD Superoxide dismutase;
TC Total cholesterol;
TG Triglyceride;
HDL-C HDL-Cholesterol;
LDL-C LDL-Cholesterol;
VLDL-C VLDL-Cholesterol

1. Introduction

Damage caused by oxygen radicals is responsible for many of the changes associated with ageing. Ageing is a progressive accumulation of physiological and morphological changes, responsible for an increasing

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*Corresponding author: Tarun Kumar Sharma, Department of Biochemistry, Pt. B.D. Sharma, University of Health Sciences, P.G.I.M.S., Rohtak (Haryana), 124001, India. Tel.: +91 989697 6598, +91 9468599091; E-mail: Sharma_bio82@yahoo.co.in.

susceptibility to disease [1]. Oxygen free radicals are implicated in the ageing process. The univalent reduction of oxygen results in a series of cytotoxic oxygen species. These highly reactive species can cause cell damage including lipid peroxidation, inactivation of enzymes, alteration of intra-cellular oxidation-reduction state and damage to DNA [2–4]. Ageing is a process of irreversible changes associated with accumulation of these oxidative damages in the cell. However free radicals do not go unchecked. Mounted against them is a multilayer defense system manned by antioxidants that react with and disarm these damaging molecules. The human body has a complex antioxidant defense system that includes enzymes like superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) and non-enzymes like glutathione (GSH) [5,6]. Since age related changes in antioxidants in erythrocytes have not been systematically studied, the objective of the present study was to evaluate the level of malondialdehyde (MDA) and antioxidants SOD, GPx, GR, CAT and G-SH in elderly people.

Blood lipid is an important indicator for health. In this study, total cholesterol, triglyceride (TG), LDL-cholesterol, VLDL-cholesterol were significantly higher while HDL-cholesterol was significantly lower in geriatric population when compared with young subjects.

Thus, screening for dyslipidemia in the elderly is recommended. On the other hand, there is no strong evidence that cholesterol lowering drugs are cost-effective in primary prevention of coronary heart disease in this group of people. So, the National Cholesterol Education Program (NCEP III, 2001) has recommended diet therapy and lifestyle modification as major strategies in lowering cholesterol level in primary prevention of coronary artery disease and limited use of cholesterol lowering drugs to only in high risk elderly who are otherwise in good health [7]. Although, a large number of epidemiological and clinical studies have analyzed the potential therapeutic utility of antioxidants [8], long-term epidemiological data showing the association of oxidative stress and mortality in humans is lacking [9]. Still, we do not know the actual importance of oxidative mechanisms such as lipoperoxidation in predicting the chance of death, either.

2. Materials and methods

The study involved 150 subjects which consisted of 75 (40 males, 35 females) healthy people between

the ages of 20–30 years as controls and 75 (40 males, 35 females) elderly subjects between the ages of 50–70 years as cases. Elderly people taking any drugs or any antioxidant supplementation were excluded from the study to avoid the possible confounding effect of such factors on lipid peroxidation and antioxidant enzymes. Subjects with diabetes, hypertension, stroke, renal failure, myocardial infarction, acute and chronic inflammatory conditions were excluded from the study. Chronic smokers, chronic alcoholics and the patients with symptomatic diseases of coronary artery disease and peripheral artery disease (atherosclerosis) were also excluded from the study. Written informed consent of all subjects was taken before initiating the study. All ethical measures were taken prior to and during the study.

2.1. Collection of blood samples

Under aseptic conditions, 4 mL of venous blood was collected from each subject. This was distributed in following vials:

- 0.4 mL in heparinized vial for estimation of reduced glutathione (GSH)
- 1.6 mL in citrated vial for estimation of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and malondialdehyde (MDA).
- 2 mL in plain vial for estimation of superoxide dismutase (SOD) and lipid profile in serum.

2.2. Preparation of haemolysate

2.0 mL blood containing citrate was centrifuged at 2000 rpm for 10 minutes. Plasma separated out for estimating MDA. The packed cell volume (PCV) was washed two times with normal saline. After that PCV was mixed gently and 1 mL of DW was added to it. Mixture was refrigerated for 10 minutes and then vigorously shaken for 12 minutes for lysis of the cell. To this 0.5 mL of chloroform was added as a preservative. Mixture was centrifuged at 3000 rpm for 20 minutes. Mixture was clearly separated into 3 layers, lower most layer of chloroform, middle of cell stroma and upper most layer of hemolysate, which was pipetted off carefully and filtered through two layers of filter paper into clear dry test tube. In this hemolysate, glutathione peroxidase, glutathione reductase and catalase were estimated.

The blood samples were analyzed spectrophotometrically for the following parameters using the standard reference methods: serum triglycerides [10], serum

Table 1
Showing the status of lipid profile, GSH, antioxidant enzymes and malondialdehyde (MDA) in geriatric and control group

Parameters	Control group <i>n</i> = 75 Median (Range)	Geriatric group <i>n</i> = 75 Median (Range)	<i>p</i> value
TC (mmol/L)	4.81 (4.2–5.98)	5.71 (4.21–6.33)	<i>p</i> < 0.05
TG (mmol/L)	1.29 (0.99–1.61)	1.36 (0.99–1.61)	<i>p</i> < 0.05
HDL-C (mmol/L)	1.24 (0.79–1.95)	1.14 (0.79–1.95)	<i>p</i> < 0.05
LDL-C (mmol/L)	3.1 (1.83–4.16)	3.65 (2.28–4.67)	<i>p</i> < 0.05
VLDL-C (mmol/L)	0.59 (0.45–0.74)	0.62 (0.45–0.74)	<i>p</i> < 0.05
GSH (mg %)	25.23 (12.39 to 35.12)	14.3 (10.0 to 18.0)	<i>p</i> < 0.001
GPx (Units/gmHb)	10.6 (8.0 to 16.0)	8.3 (6.2 to 11.0)	<i>p</i> < 0.001
GR (Units/gmProtein)	18.7 (18.0 to 20.8)	16.6 (10.1 to 18.3)	<i>p</i> < 0.001
CAT (Units/gmProtein/mL)	5.3 (4.0 to 7.0)	5.8 (4.9 to 7.0)	<i>p</i> < 0.001
SOD Units/mgProtein/mL)	8.4 (6.1 to 10.7)	6.5 (4.3 to 8.9)	<i>p</i> < 0.001
MDA (nmol/mL)	3.3 (2.0 to 6.0)	5.1 (3.0 to 6.0)	<i>p</i> < 0.001

p < 0.001, Significant.

total cholesterol [11], serum HDL-cholesterol [12]. Serum LDL-cholesterol and serum VLDL-cholesterol were calculated by the Friedewald formula [13]. Reduced glutathione [14], glutathione peroxidase [15], glutathione reductase [16], catalase [17] were estimated in erythrocytes. Superoxide dismutase was estimated in serum [18]. Malondialdehyde was estimated in plasma [19]. The samples were stored at +2°C to +8°C (+4°C) before analysis and all the samples were analysed on the same day of collection.

Reduced Glutathione (GSH) level was determined according to Beutler et al. [14] in a meta-phosphoric acid filtrate by using 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). Enzymatic activity was expressed in mg %. Mg % of glutathione in blood in three coleman junior instruments identical E values were found that is 0.542.

Glutathione peroxidase (GPx) activity was determined according to Hafeman et al. [15] by using hydrogen peroxide as a substrate. Enzymatic activity was expressed in U/gmHb.

Glutathione reductase (GR) was determined according to Horn et al. [16] which catalyses the reduction of GSSG in the presence of NADPH which is oxidized to NADP⁺. Enzymatic activity was expressed in Units/gm Protein.

Catalase (CAT) was estimated by the method of Sinha [17] and was assayed colorimetrically at 620 nm. The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H₂O₂), with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured colorimetrically. Catalytic activity was expressed in Units/gm Protein/mL.

The activity of superoxide dismutase (SOD) was assayed by applying the method of Mishra et al. [18]. The ability of superoxide dismutase to inhibit the auto-oxidation of epinephrine at pH 10.2 has been used as

the basis of a convenient and sensitive assay for this enzyme. Enzymatic activity was expressed in U/mg Protein/mL.

Plasma malondialdehyde (MDA) was estimated by method of Jean et al. [19]. After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was extracted in butanol and was measured. Enzymatic activity was expressed in nmol/mL.

2.3. Statistical analysis

All results were expressed in Median (Range). Differences between the Median (Range) were calculated by Mann-Whitney U test. The statistical analysis was performed using SPSS version 17.0. Pearson correlation coefficients were used to analyze relationships between biochemical parameters. The level of significance was set as *p* value < 0.001 to be significant and *p* value > 0.05 as non-significant.

3. Results

All results were expressed in Median (Range) (Table 1). In our study we observed a significant decrease (*p* < 0.001) in reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD) in geriatric population as compared to the control group. However there is a significant increase (*p* < 0.001) in the level of catalases (CAT) and malondialdehyde (MDA).

We also observed a significant decrease (*p* < 0.05) in (HDL- C) high density lipoprotein cholesterol and there is a significant increase (*p* < 0.05) in total cholesterol (TC), triglyceride (TG), LDL-cholesterol (LDL-C), VLDL-cholesterol (VLDL-C).

The Pearson correlations data between all the study parameters with lipid profile, GSH, antioxidant en-

Table 2

Showing the Pearson correlations data between all the study parameters with lipid profile, GSH, antioxidant enzymes and MDA in geriatric population

Parameters	TG	HDL-C	LDL-C	VLDL-C	GSH	GPx	GR	CAT	SOD	MDA
TC	$r = 0.49$ $p < 0.05$	$r = -0.32$ $p < 0.05$	$r = 0.97$ $p < 0.05$	$r = 0.61$ $p < 0.05$	$r = -0.39$ $p < 0.05$	$r = -0.30$ $p < 0.05$	$r = -0.48$ $p < 0.05$	$r = 0.24$ $p < 0.05$	$r = -0.42$ $p < 0.05$	$r = 0.42$ $p < 0.05$
TG	—	$r = -0.33$ $p < 0.05$	$r = 0.39$ $p < 0.05$	$r = 0.72$ $p < 0.05$	$r = -0.56$ $p < 0.05$	$r = -0.45$ $p < 0.05$	$r = -0.56$ $p < 0.05$	$r = 0.23$ $p < 0.05$	$r = -0.37$ $p < 0.05$	$r = 0.55$ $p < 0.05$
HDL-C	—	—	$r = -0.39$ $p < 0.05$	$r = -0.43$ $p < 0.05$	$r = 0.42$ $p < 0.05$	$r = 0.33$ $p < 0.05$	$r = 0.40$ $p < 0.05$	$r = -0.24$ $p < 0.05$	$r = 0.26$ $p < 0.05$	$r = -0.30$ $p < 0.05$
LDL-C	—	—	—	$r = 0.46$ $p < 0.05$	$r = -0.35$ $p < 0.05$	$r = -0.27$ $p < 0.05$	$r = -0.41$ $p < 0.05$	$r = 0.22$ $p < 0.05$	$r = -0.40$ $p < 0.05$	$r = 0.36$ $p < 0.05$
VLDL-C	—	—	—	—	$r = -0.56$ $p < 0.05$	$r = -0.41$ $p < 0.05$	$r = -0.64$ $p < 0.05$	$r = 0.31$ $p < 0.05$	$r = -0.37$ $p < 0.05$	$r = 0.52$ $p < 0.05$
GSH	—	—	—	—	—	$r = 0.54$ $p < 0.05$	$r = 0.57$ $p < 0.05$	$r = -0.094$ $p > 0.05$	$r = 0.54$ $p < 0.05$	$r = -0.044$ $p > 0.05$
GPx	—	—	—	—	—	—	$r = 0.62$ $p < 0.05$	$r = -0.183$ $p > 0.05$	$r = 0.52$ $p < 0.05$	$r = 0.121$ $p > 0.05$
GR	—	—	—	—	—	—	—	$r = -0.182$ $p > 0.05$	$r = 0.46$ $p < 0.05$	$r = -0.023$ $p > 0.05$
CAT	—	—	—	—	—	—	—	—	$r = 0.155$ $p > 0.05$	$r = -0.162$ $p > 0.05$
SOD	—	—	—	—	—	—	—	—	—	$r = -0.037$ $p > 0.05$

Correlation is significant at 0.05 levels (2- tailed); $p > 0.05$, Non-significant; $p < 0.05$, Significant.

zymes and MDA in geriatric population is shown in Table 2.

The significant negative correlation were observed in HDL-C, GSH, GPx, GR and SOD with TC and TG; LDL-C, VLDL-C, CAT and MDA with HDL-C; GPx, GR and SOD with LDL-C and the significant positive correlation in LDL-C, VLDL-C, CAT and MDA with TC and TG; GSH, GPx, GR and SOD with HDL-C; VLDL-C, CAT and MDA with LDL-C in geriatric population ($p < 0.05$, Table 2).

The significant positive correlation were observed in GSH with GPx, GR and SOD; GPx with GR and SOD; GR with SOD ($p < 0.05$, Table 2).

The non-significant negative correlation were observed in CAT with GSH, GPx, GR and MDA and positive correlation with SOD. Also non-significant negative correlation were observed in MDA with GSH, GR and SOD and positive correlation with GPx in geriatric population ($p > 0.05$, Table 2).

4. Discussion

The oxidative stress theory of aging offers the best mechanistic elucidation of the aging phenomenon and other age-related diseases. Susceptibility of the oxidative damage mechanisms operating in the body which are induced to maintain the antioxidant capacity during aging [20]. The evidences of potential beneficial role of exogenous antioxidants in preventing atherosclerotic

disease are ambiguous. Although experimental evidences strongly suggest that oxidized LDL accelerates atherosclerosis, the epidemiologic evidences from clinical trials are not entirely consistent with the potential role of exogenous antioxidants in preventing atherosclerotic disease [21].

Aging is associated with changes in physical characteristics and decline of many physiological functions. The excessive production of free radicals in the organism, and the imbalance between the concentrations of these and the antioxidant defenses may be related to aging [22,23]. Oxidative stress results from the imbalance between oxidative and antioxidative mechanisms with increased levels of pro-oxidants and depletion of antioxidants leading to tissue damage. MDA is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polyunsaturated fatty acids of biological membranes. In the present study, we found serum MDA levels were significantly higher in the group of elderly persons, as compared with the younger group.

Lipid peroxidation occurs as a result of oxidative destruction of polyunsaturated fatty acids (PUFAs) located in cell membranes that are readily attacked by oxidizing radicals [24]. The lipid peroxidation proceeds as self-perpetuating chain reaction to form lipid peroxides and aldehydes like MDA which are cytotoxic and mutagenic [25]. Moreover, lipid peroxidation has been implicated in a wide range of tissue injuries and diseases e.g. atherosclerosis and coronary artery disease.

The present study shows that levels of GSH, SOD, GPx and GR were significantly decreased and that of CAT was increased. The antioxidant enzyme superoxide dismutase (SOD), the most important enzyme present virtually in all aerobic organisms, catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Glutathione peroxidase is a selenoenzyme which catalyzes the reduction of hydroperoxides at the expense of reduced glutathione. Catalase is a primary antioxidant defense component that works to catalyze the decomposition of hydrogen peroxide to water, sharing this function with GPx [26].

The hypothesis that the antioxidative capacity is lowered in older subjects is supported by our data for SOD. This is in line with findings of other studies indicating that older subjects had lowered SOD activities [27,28]. A decrease of SOD with aging might be due to a lowered metabolic rate and oxygen consumption generating less ROS, and thereby a lower demand for SOD.

SOD and GPx concentrations are reduced as they continuously work to neutralize the superoxides, hydrogen peroxide and hydroperoxides produced in increased amount after menopause. Levels of CAT were observed to be increased which may be explained by the fact that the activity of catalase increases when the level of GPx decreases because they both work together to perform similar task of neutralizing hydrogen peroxide [29]. Proper antioxidants plus an appropriate diet, have health benefit to reduce morbidity and perhaps increase the healthy, useful life span of an individual [30].

In this study, total cholesterol, triglyceride (TG), LDL-cholesterol, VLDL-cholesterol were significantly higher while HDL-cholesterol was significantly lower in geriatric population when compared with young subjects. It has been concluded that in aging men low serum testosterone concentration is associated with a potentially atherogenic lipid profile, characterized by low HDL cholesterol and high triglycerides concentrations [31].

A number of trials have indeed shown a clear association between dyslipidemia and intima-media thickness (IMT) [32,33]. The ageing process by itself causes changes both in the architecture and constitution of the vascular wall. The aging endothelium releases less nitric oxide (NO), which is important for vascular relaxation. The collagen increases, whereas elastin becomes progressively disrupted, thinner, and often fragments itself. Deposits of fat and calcium are formed, with concurrent loss of elasticity. These aging-related vascular changes have important medical implications in CVD pathogenesis [34]. On the other hand, evi-

dence that hyperlipemia leads to carotid IMT [35] has triggered a number of studies with ultrasound assessment, which have shown a positive relationship between carotid IMT and LDL-C, even in elderly subjects. In regards to dyslipidemia, observation studies in elderly populations have showed contradictory results. O'Leary and colleagues (1996) investigated a population of 5117 subjects with 65 years or older, and found a positive relation between high levels of LDL-c and IMT, while high levels of HDL-c presented a negative relationship [36]. Bonithon-Kopp and associates (1996) observed a positive relationship between cholesterol levels and presence of plaques in the carotid arteries; however, no relationship was found for IMT [35].

5. Conclusion

It is evident from this study that there is enhanced oxidative stress and decreased antioxidant defence in elderly group as compared to young subjects which can play an important role in ageing. In conclusion, the antioxidant defense mechanisms are not sufficient to prevent age related increase in oxidative damage and additional dietary intake of a variety of antioxidants might be beneficial for preserving the normal function in elderly people. Therefore antioxidants in the form of micronutrients and vitamins may be given as supplements in elderly people.

Future studies will need to address targeting therapy to specific tissues and finding appropriate markers for functional consequences of the attenuation of oxidative stress in aging.

As patients grow older, they are more likely to have multiple chronic diseases, including CVD, cerebrovascular disease, kidney disease, diabetes, and metabolic syndrome. Older patients also are more likely to have decreased hepatic function and decreased muscle mass. These factors can all influence screening and treatment decisions in elderly patients.

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Conflict of interest

The authors declare that they have no competing interests.

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