Vicissitudes in Lipid Peroxidation and Antioxidants in Elderly Niddm Patients

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Abstract: **Advanced age in type-II diabetic patients is associated with an accumulation of free radical damage, which leads to physiological and\ clinical modifications. Age related changes resulting from free radical reactions include increasing levels of lipid peroxides, alterations in enzyme activities and greater osmotic fragility. The present study was conducted to estimate the level of lipid peroxidation product – Malondialdehyde and antioxidants Catalase and Glutathione in elderly people with Non Insulin Dependent Diabetes Mellitus. An increase in lipid peroxidation and decrease in antioxidants was observed in these people. Highly significant increase in MDA and decrease in antioxidants was observed. When complicated with diabetes and hypertension. Supplementation of antioxidants may prevent further oxidative injury in these patients.**

***Keywords- Lipid peroxidation, Antioxidants, NIDDM elderly patients*.**

I. INTRODUCTION

Demolishing proteins and damaging nucleic acids, oxygen radicals are thought to be the villains in the day-to-day life of cells. Damage caused by oxygen radicals is responsible for many of the bodily changes that come with ageing and diabetes. Ageing is a progressive accumulation of physiological and morphological changes, responsible for an increasing susceptibility to disease [1]. Oxygen free radicals are implicated in the ageing process especially with diabetic patients. 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. But free radicals do not go unchecked.

Mounted against them is a multilayer defense system manned by anti-oxidants 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 (G-SH Px), catalase (CAT) and non-enzymes like vitamins A, E, C and glutathione (G-SH) (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 lipid peroxidation (MDA) and antioxidants CAT and G-SH in elderly NIDDM patients.

II. MATERIALS AND METHODS

The study group included 91 elderly people selected without known bias between 60-75yrs of both sexes from LLR Hospital, GSVM Medical College, Kanpur, and Rama Medical College, Kanpur. All subjects gave written consent before the beginning of the study. Information regarding chronic illness, smoking, alcohol consumption and drug intake was obtained by questionnaires. They were divided into four groups: [1] normal elderly group, n= 13, [2] those with diabetes, n= 22, [3] those with hypertension, n= 19 and [4] those with both diabetes-hypertension, n=37. The control group included 15 healthy individuals of both sexes between 20- 32yrs. Random venous blood samples were collected into heparinized bottles. 0.2ml of the whole blood was used for the assay of Glutathione (G-SH). Rest of the blood was centrifuged within 3hrs of collection at 3000rpm for 10mins. Plasma was discarded. RBC's were mixed with 0.9% saline and centrifuged. Supernatant was removed. The process was repeated 3 times to prepare RBC suspension which was used for the assay of Malondialdehyde (MDA) and Catalase (CAT). MDA in the RBC was estimated by the modified method of Stocks and Dormandy by TBA reaction [7]. Values were expressed as nanomoles per deciliter. The catalase activity of the hemolysate was determined by adopting the method of [8-9]. Values were expressed as Units per gram hemoglobin. The hemoglobin content of the erythrocytes was determined by cyanmethemoglobin method (10). G-SH concentration was measured by the method of Ernest Beutler [11]. Values were expressed as milligrams per deciliter. Mean and standard deviation was calculated separately for all the groups and compared across the groups using Kruskal-Wallis test. Post-hoc comparison was done and significance derived for each pair wise comparison. 'p' value < 0.05 was considered statistically significant.

III. RESULTS

The increase in MDA is significant in normal elderly people ($p<0.05$) and elderly hypertensive patients ($p<0.001$) and in elderly diabetic patients ($p<0.001$) when compared to normal young subjects (Table 1). The MDA levels are very highly increased ($p<0.0001$) in elderly diabetic-hypertensive patients when compared to normal young subjects (Table 1). The G-SH levels are increased in normal elderly group when compared to normal young subjects ($p<0.05$) where as it is highly decreased in elderly hypertensive, elderly diabetic and elderly diabetic-hypertensive patients ($p<0.0001$) when compared to the normal young controls (Table1

Table 1: COMPARISON OF MDA, GSH AND CATALASE LEVELS IN ALL THE STUDY GROUPS WITH THAT OF NORMAL YOUNG CONTROLS (MEAN ± SD)

	Normal Young n=15	**Normal Elderly n=13**	**Elderly Hypertensive n=19**	**Elderly Diabetic n=22**	**Elderly Diabetic Hypertensive n=37**
MDA	352.26 ±67.59	396.39± 43.58	551.16± 199.52	555.87± 88.39	749.42 ±260.61
'p' value		<0.05	<0.001	<0.001	<0.0001
GSH	50.08± 5.93	56.45±8.81	35.15±3.88	36.59±3.69	37.02± 2.34
'p' value		<0.05	<0.0001	<0.0001	<0.0001
CAT	1,25,048±30,794	48,030± 24,002	85,422± 39,258	1,14,313 ±53,415	82,201 ±22,572
'p' value		<0.0001	<0.05	>0.05NS	<0.05

n = number of samples SD = standard deviation NS = Not significant
'p' value = probability of chance being cause for difference in mean of the two groups.

IV. DISCUSSION

Our results indicate that there is increase in free radical generation and decrease in antioxidant defense mechanism in elderly people and diabetic elderly patients, when compared to normal young subjects. Highly significant increase in MDA and decrease in antioxidants was observed in elderly people when complicated with diabetes and hypertension. A negative correlation was obtained between MDA and antioxidant GSH. Increased MDA and decreased antioxidants with ageing indicate that peroxidative damage increases with ageing process. Lipid peroxidation is an autocatalytic process, which ultimately results in cell death [6]. Because of continuous generation of free radicals by the oxidation of haemoglobin, erythrocytes are exposed to continuous oxidative stress. It has long been known that there is an imbalance between oxidants and antioxidants in diabetes mellitus [12]. Lipid peroxidation (MDA) levels of diabetic and hypertensive patients are higher than in normal young people as seen in our study. The reason for increased MDA levels in diabetes mellitus may be due to increased reactive oxygen products and decreased antioxidants. Reactive oxygen metabolites including free radicals increase the auto-oxidation of glucose and glycosylated proteins in diabetes mellitus and the activation of the sorbitol pathway during hyperglycemia [13-15]. Insufficient neutralization of free radicals causes the oxidation of cellular lipids, proteins, nucleic acids, glycolipids and glycoprotiens [15]. This oxidative effect also causes damage to the vascular endothelial cells, as evident from increased MDA levels observed in our study in elderly diabetic and hypertensive patients. The increased levels of lipid peroxides can cause oxidative injury to blood cells, cross linking of membrane lipids and proteins, imbalance of prostacyclin, prostaglandin and vasoconstriction. Our study also shows that there is increase in MDA levels in the elderly hypertensive patients when compared to all the other groups. Elevation of oxidative stress and associated oxidative damages are mediators of vascular injury in various cardiovascular pathologies including hypertension, atherosclerosis, ischemia-reperfusion and diabetes. Reactive oxygen species (ROS) are biologically important oxygen derivatives that are recognized to be important in vascular biology through their oxidation/reduction (redox) potential. All vascular cell types (endothelial cells, vascular smooth muscle cells and adventitial fibroblasts) produce ROS primarily via cell membrane associated NADPH-oxidase. ROS regulate vascular function by modulating cell growth, apoptosis, migration, inflammation, secretion and extracellular matrix production. An imbalance in redox state where pro-oxidants over whelm antioxidant capacity results in oxidative stress. This oxidative stress causes the vascular injury and inflammation in cardiovascular diseases, hypertension and diabetes. Prostaglandin synthesis is affected due to oxidation of Arachidonic acid, a polyunsaturated fatty acid (PUFA). Therefore it is claimed that the long term complications of diabetes mellitus and hypertensive patients are related to the accumulation of increased free radicals and lipid peroxidation products. The present study also indicates age related decrease in glutathione (G-SH) activity in elderly people complicated with diabetes and hypertension. A negative correlation was obtained between MDA and G-SH. A slight increase in G-SH level was observed in elderly group without any health complications. The tripeptide glutathione (gamma -L-glutamyl -L- cysteinylglycine) is the major intracellular non-protein thiol compound and plays a major role in the protection of cells and tissue structure [18]. The G-SH antioxidant system is the body's powerhouse for diffusing and disposing of radicals that threaten the cell, tissue and organ damage, thus slowing the approach of age. Cells depleted with intracellular G-SH were more susceptible to oxidant mediated cell death through the dysregulation of the glutathione antioxidant system. There are several possibilities for the occurrence of lower G-SH concentration in erythrocytes of aged persons complicated with diabetes and hypertension. These include increased rate of oxidation in senescence [19], decreased G-SH synthesis due to cysteine deficiency [20] and/or diminished activity of gamma glutamylcysteine synthetase [21] and increased GSH consumption in the removal of peroxides and xenobiotics [22-23]. High blood levels of G-SH predict good health whereas, low levels predict early disease. In our study we have also found that erythrocyte

catalase activity is highly decreased in normal elderly subjects when compared to normal young subjects; whereas it is slightly reduced in other groups. Decreased activity of catalase with ageing might be due to its inactivation by increased oxidative stress. These findings show that antioxidant activities are affected with ageing and there was age related lipid peroxidation. This free radical mediated peroxidative injury has a role in pathophysiological changes of ageing. In conclusion, the antioxidant defense mechanisms are not sufficient to prevent age related increase in oxidative damage and dietary intake of a variety of antioxidants might be beneficial for preserving the normal function both in elderly people and NIDDM elderly patients.

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