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Research Article

STUDIES OF DIABETES IN ELDERLY FEMALES

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ABSTRACT

The objective of the study was to evaluate the effect of Diabetes in elderly females. Oxidative stress is regarded as a main causal factor for natural ageing. This study tested the hypothesis that healthy elderly people show higher oxidative DNA damage levels and lower antioxidative enzymatic defense capacities. In type II diabetic patients, persistence of hyperglycemia is one of the causes of increased in oxygen free radicals. This increase oxidative stress and makes the life threatening cardiovascular complications. Blood samples were drawn from selective 100 elderly females (with and without diabetes). In elderly females having menopause, there occur hypercholesterolemia, hypertriglyceridemia, and hyperlipoproteinemia, these on the body, escort an increase in free radicals, with mounting oxidative stress. In diabetes the body suffers with a poor or negligible glycemic control. All diabetic post menopausal females with CVD have been reported a significant high levels of Fasting Blood Sugar, Total Cholesterol, Triglycerides, LDL-C, VLDL-C, CAT, NT-proBNP and MDA and significantly lower levels of HDL-C, GPx, SOD as compared to control subjects. In type-II diabetes mellitus elderly female patients during the postmenopause, the probability of secondary complications of cardiovascular diseases increases.

Key words: Antioxidant Enzymes, Cardiovascular diseases, Diabetes Mellitus type-II, Estrogen and NT-proBNP (Amino Terminal –pro Braintype Natriuretic Peptide).

INTRODUCTION

Oxidative stress is regarded as a main causal factor for natural ageing. Different hypotheses have been proposed to explain the aging processes in humans. Among the related theories, the most advanced is the oxidative damage theory, which has been developed from the free radical theory of Harman[1]. It considers oxidative stress as main causal factor for natural aging and is based on the high reactivity of reactive oxygen species (ROS), which are ubiquitously generated byproducts of the oxygen metabolism in living beings[2]. The healthy elderly people show higher oxidative DNA damage levels and lower antioxidative enzymatic defense capacities than younger ones. In contrast to this few other studies reported, older and younger subjects showed moderate to medium physical activity, but not exhaustive activity.

The older subjects of both sexes displayed a somewhat higher level of physical activity than younger ones, but age group differences were statistically nonsignificant. Thereby, the physical activity level of the subjects was not associated with altered oxidative stress level, oxidative DNA damage, or antioxidant enzyme activities[3]. Oxidative stress is caused in humans by various pathophysiological mechanisms, in particular by the increased production of ROS during inflammatory processes and by various environmental factors (ionizing radiations, nicotine, alcohol, unbalanced diet, etc.). Protective enzymatic and non-enzymatic antioxidant defense mechanisms reduce oxidative stress by degradating ROS. Major intracellular antioxidative enzymes are the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). They act in two steps: firstly, SOD converts the highly active superoxide radicals (**.**O2 -) into hydrogen peroxide (H2O2) and oxygen (O2). Afterwards, CAT and GPx independently convert H2O2 to water and oxygen[4]. Glutathione (GSH) is not only a cofactor for GPx, but can also react as a direct scavenger of ROS[5]. On the non-enzymatic level, also vitamins (vitamin C, vitamin E and β carotene) and other antioxidant compounds scavenge free radicals and delay oxidation of molecules.

While antioxidant defense mechanisms inhibit damage generation, DNA repair enzymes (glycosylases) remove DNA base modifications generated by ROS. One of the most abundant DNA lesions resulting from reaction of ROS with DNA is 7,8-dihydro-8-oxo-2'-deoxyguanosine (8-oxo-dG), a modification caused by hydroxylation of the C-8 position of guanine[6]. It causes a Guanine →Thymine (G→T) transversion because of its mispairing with adenine[7]. Increases of 8-oxo-dG with age have been observed by several groups, in human brain[8]. Oxidative status was increased in older men and negatively correlated with glutathione concentrations. GPx activity was elevated and the SOD/GPx ratio lowered in older males. Subjects with lowered SOD/GPx ratio showed increased oxidative DNA damage. They indicated, age-related changes in the balance between first step (SOD) and second step (GPx) of the enzymatic antioxidant defense system. They support the assumption that a biological optimum between antioxidative enzymes might be more important than their absolute activities. In humans, an excess of ROS has been made responsible for premature aging and the development of atherosclerosis and cancer[9]. Increased oxidative stress has also been found in patients with chronic renal failure. The depletion of defensive body chemicals (antioxidants) increases the risk of complications during diabetes.

Diabetes mellitus, a major health problem, is a group of metabolic disorders characterized by hyperglycemia and glycosuria with disturbances in carbohydrate, fat and protein metabolism resulting from either an absolute or relative deficiency of insulin secretion or action. The most common type of diabetes is type-II Non insulin dependent diabetes mellitus (NIDDM). Diabetes mellitus is also characterized by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation[1] due to decreased biological response to insulin. A combination of insulin resistance and beta cell failure leads to diabetes mellitus type-II. There occurs persisting hyperglycemia, leading to enhanced auto oxidation of glucose, which is one of the major causes for generating oxidative stress. Factors like polyol pathway, prostanoid synthesis and protein glycation further increase the uproar in the antioxidant defense system of cells, via increasing the functional activities and formation of superoxide anion ($O2^{-\cdot}$), the hydroxyl radical (OH^{-}.) and hydrogen peroxide (H_2O_2). In diabetes, equilibrium between pro-oxidants and antioxidants is disturbed, which causes alterations in the metabolism of body, and further resulting in enhanced progressive development of microvascular complications like retinopathies, nephropathies, neuropathies. Elevated lipid peroxidation products, malonaldialdehyde (MDA) and reduced glutathione (GSH) levels have been mixed up in the pathophysiology of type-II diabetes mellitus.

Normally, free radicals are formed in minuscule amount and are rapidly scavenged by natural cellular defense mechanisms comprising of enzymes like Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Glutathione reductase (GR), Catalase (CAT) etc. An increased production of MDA, a marker for lipid peroxidation has been found in RBCs membrane of diabetics along with low red blood corpuscles' (RBCs) contents i.e. antioxidant enzymes and

reduced glutathione (GSH). An exaggerated oxidative stress has been postulated as the link between hyperglycemia and cardiovascular diseases. Hyperglycemia, the primary root cause of type-II diabetes, has been accepted as being essential for the development of diabetic cardiovascular complications.

Lipid peroxidation is a free radical-related process, which is potentially harmful because its uncontrolled, self-enhancing process causes disruption of membranes, lipids and other cell components. Some complications of diabetes are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products[10]. Free radicals are naturally produced *in vivo*, both by normal cellular metabolism and as a result of disease process or through xenobiotic activities. They have potential to elicit various tissue changes associated with toxicities and disease processes, but are also a consequence of such damage[11].

Lipid peroxidation is found to be involved in oxidative stress, which plays a major role in the pathogenesis of diabetic mellitus[12]. The major role of the antioxidants is to protect, prevent and/or reduce the extent of oxidative destruction of cellular tissues. Increased free radical production is said to medicate tissue injury in a wide range including diabetes mellitus and other cardiovascular diseases[13]. The organism's susceptibility to free radical stress and peroxidative damage is related to the balance between the free radical load and the adequacy of antioxidant defenses. Abnormally high levels of lipid peroxidation products and the simultaneous decline of antioxidant defense mechanisms is usually harmful due to its disruption of membrane lipid leading towards damage of cell-organelles, and increase in oxidative stress. Very little information is available, about the effect of antioxidant, and vitamins, on oxidative stress indices, such as MDA, lipid peroxidation, and levels of glutathione in type -II diabetic elderly[14].

Menopause is the term for the end of a women menstrual period. During ageing, after the age of 40s, the most common feature amongst females is menopause. When ovaries stop making estrogen hormone, there occurs menopause. Thus estrogen level secretion is decreased, which leads to the end of monthly menstrual periods that have both significant long term and short term consequences. Estrogen hormone has cardioprotective functions, and a potent antioxidant. The deficiency or absence of this in menopausal females leads to increase in oxidative stress in body. The diabetic postmenopausal females become prone for various cardiovascular complications. Potentially proatherogenic changes in the serum lipid profile have been associated with menopause including an increase in total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) whereas decrease in high density lipoprotein cholesterol (HDL-C). Menopause is correlated with ageing and deficiency of estrogen. According to free radical theory of ageing, the activity of antioxidant enzymes is altered[13]. Moreover, the deficiency of estrogen in menopausal women causes, increase in lipid peroxidation which subsequently leads to depletion of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase. This study denotes the relationship between levels of serum lipid peroxidation product, malondialdehyde, an oxidant, reduced glutathione level in menopausal elderly diabetic females.

MATERIALS AND METHODS

The study was conducted on selective 100 cases (25 female, and 25 male) of NIDDM patients. This consisting of 25 postmenopausal females (45-60yrs) without any diabetic complication were opted as control, 25 age-matched diabetic post-menopausal females with cardiovascular complications were selected from patients regularly attending our health centre for their monthly routine medical examination. Simultaneously we selected 25age-matched elderly NIDDM males and 25 non-diabetic healthy elderly males without any cardiovascular complications. All subjects had not been taking any medicines other than antidiabetic pills since, past 3-5 years, were having normal hepatic, endocrine functions. Patients with heart disease, renal insufficiency, hypertension, and other life-threatening diseases, as cancer, were excluded. Reagents used were from Roche and Bayer Diagnostics India Ltd. The fasting venous blood was drawn from diabetic patients and healthy volunteers around 8:00am and the serum was

separated immediately by centrifugation at 3000g for 15 min using cooling research centrifuge. In diabetic and nondiabetics, the protein contents, Lipid peroxidation product, MDA, Glutathione (GSH) and Glutathione reductase (GR) levels, Fasting blood sugar (FBS), Catalase(CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GPx),NT-proBNP, TroponinT, HbAc1 and Lipid profile etc. were estimated by the standard procedures as described elsewhere by standard methods. These biochemical parameters were determined by spectrophotometer, using double beam (UV-2101, spectrophotometer from Shimadzu, Japan). Data of normal and diabetic patients were compared by ANOVA followed by Student's t-test.

RESULTS

The male and female type-II diabetic patients had significantly elevated levels of fasting blood glucose, whereas the subjects without diabetic complications as control. The study groups were well for sex and age-matched with their respective control groups. There were no significant differences in triglycerides, all diabetic post-menopausal females with cardiovascular diseases had significantly higher levels of Fasting blood sugar (FBS), TC, TG, LDL-C, VLDL-C, Catalase (CAT) and Malondialdehyde (MDA) and significantly lower levels of HDL-C, GPx, SOD as compared to those in control subjects, whereas reduced Glutathione (GSH) change was not significant. Total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol in diabetic male/female patients, were compared with controls. The serum MDA level, increased significantly, whereas the reduced glutathione and protein content decreased significantly in male and female diabetic patients in comparison to non-diabetics.

DISCUSSION

Oxidative stress plays a major role in the pathogenesis of type-II diabetes mellitus. Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which plays an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and formation of several damages in diabetes mellitus. We observed that MDA level, were elevated significantly in both male and female diabetics. The diabetic patients, irrespective of the sex, were exposed to an increased oxidative stress via lipid peroxidation. The other researchers have also reported elevated lipid peroxidation products in blood samples of type I and type-II diabetic patients[15-16]. Abnormally-high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defense mechanisms can damage cellular organelles and enzymes. These consequences of oxidative stress can promote the development of complications in diabetes mellitus patients. Antioxidant enzyme-dependent defense play an important role in scavenging free radicals produced under oxidative stress[17]. Glutathione, an antioxidant, and the protein content of blood serum of diabetic mellitus patients was significantly low, indicating decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid peroxidation processes. Menopausal diabetic women suffered from deficiency of estrogen and insulin, both are considered as cardioprotective hormone. The estrogen act as antioxidant, the deficiency of which causes a decreased or inactivity of antioxidant enzymes of RBC resulting in increased fragility of RBC. Sometime the ageing is associated with anemia, which may be due to the above reasons. Apart from these, socio-economic and psychological factors, also influences the body in the older stages of life.

Menopause and Emotions: People once mistakenly thought depression and moodiness were to be expected of women going through their change of life. Although we now know it's a myth that women can "go mad" during menopause, most women do report emotional changes as their hormone levels fluctuate and readjust, even so far as describing their experience as an emotional rollercoaster. Long-standing emotional difficulties may worsen during menopause. However, it does women an injustice simply to point the finger directly at their biochemistry. Research studies in the physiology and psychology of women as they proceed along the life span show that these conditions have varied causes. Emotions triggered by hormonal changes often

are intensified by other changes in your life, and vice versa. During menopause a female may experiences stresses in her social world as children leave home, parent dies, or change in marital status. They may feel out of place in their own youth-oriented society; may mourn the loss of the reproductive capacity; or may feel new pressures on the job. They may feel diminishing self-esteem and social power (sometimes culture devalues older people, and older women most of all.) Fortunately, natural therapies are effective tools in learning to cope successfully with the psychological and mental aspects associated with menopause, as well as the physical ones. Further work may be undertaken to confirm the association between antioxidant nutrient intake and the reduction in the development of cardiovascular complications along with these factors. Most of the antioxidant enzymes are metalloenzymes and any deficiency of micronutrients may also become a cause of decreased or malfunctioning activity of antioxidant enzymes. All diabetic post-menopausal females with cardiovascular diseases had significantly higher levels of FBS, PPBS, TC, TG, LDL-C, VLDL-C, Catalase, and MDA, due to continuous generation of free radicals by the oxidation of hemoglobin, erythrocytes are exposed to continuous oxidative stress[18]. The erythrocytes antioxidant enzyme activities and peroxidative injuries were observed in elderly female patients. A significant decrease in glutathione concentration in diabetic women as compared to control group was observed, which may be due to an increase lipid peroxidation and non-availability of the substrates, i.e. Cysteine, Glutamine, Glycine, etc. We observed that erythrocyte GPx activity was very low in patients suffering from cardiovascular disease compared with control subjects[19]. The low activity of GPx could be directly explained by the low content of GSH found in diabetic patients with cardiovascular disease, since GSH is a substrate of GPx[20]. Enzyme inactivation also contributes to low GPx activity. GPx is a relatively stable enzyme, but it may be inactivated under conditions of severe oxidative stress. This may occur through glycation governed by prevailing glucose concentration. The low activity of GPx causes accumulation of H_2O_2 in RBC of diabetic patients. These findings could also explain the progressive decrease in Superoxide dismutase in later stages of the diabetes. In diabetic patients, the autooxidation of glucose results in the formation of hydrogen peroxide, which inactivates Superoxide dismutase. Therefore the accumulation of H_2O_2 may be one of the explanations for decreased activity of Superoxide dismutase in these patients. The activity of Catalase decrease with ageing suggests an increase in H_2O_2 formation. During the process of ageing steady-state concentrations of erythrocytes, H_2O_2 may be much higher, which could lead to induction of the Catalase activity[21]. Diabetic humans had shown increased lipid peroxidation and decreased levels of reduced glutathione, glutathione reductase, glutathione peroxidase, glutathione and G6PDH. Serum TBARS levels were increased, but no significant changes in superoxide dismutase (SOD) activity was observed in type 2 diabetic patients.

MDA levels in our study were increased with ageing in postmenopausal group of women. Our findings show that peroxidative damage increases with the ageing process, which indicated that plasma MDA levels were increased with age in healthy subjects[22-23]. Increase oxidative stress as measured by the markers of oxidative stress[24] has been shown to be increased in diabetes-II leading to onset and progression of further late-diabetes complications[25]. Usually the measurement of oxidative stress is based on indirect measurement of free radicals. The levels of these free radicals are controlled by levels of antioxidant enzymes as well as non-enzymatic scavengers like reduced GSH, vitamins, selenium and others. Accumulation of lipid peroxidation products occur during ageing[26]. Malondialdehyde, one of the lipid peroxidation products is frequently used to determine the oxidant/antioxidant balance in diabetic patients[27]. Hyperglycemia has been shown to cause permanent alteration in proteins and lipid peroxidation in a variety of experimentally streptozotocin-induced diabetes[28]. Hyperglycemia itself may stimulate platelet aggregation and autooxidation of glucose which may result in free radical production[29]. The reduced glutathione and uric acid are physiological scavengers. Glutathione plays a central role in antioxidant defense. Reduced glutathione maintains the integrity of RBCs membrane and regenerates the major aqueous and lipid phase antioxidants i.e., as ascorbate and α–tocopherol.

It has been shown to be a primary agent involved in redox regulation of protein thiols. In hyperglycemic conditions, glucose is preferentially used in the polyol pathway that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme[30-32]. Hyperglycemia is therefore indirectly the cause of GSH depletion and this result in oxidative stress. The lipid profile status is to be correlated with menopausal diabetic women since the lipid profile status is maintained by estrogen and insulin. The incidences of cardiovascular diseases are increased in this group, as shown in our study also. Along with this, there occurs a decrease in concentration of HDL-C while other parameters are increased.

In menopausal women ageing along with diabetes causes severe changes, due to deficiency of insulin and estrogen rather than independently associated with ageing. These results also suggest that the increase in lipid peroxidation and the decline in antioxidant defenses may appear early in type-II NIDDM patients, before the development of secondary complications. Studies had also observed that long term vitamin E supplementation could reduce the morbidity and mortality rates associated with complications in diabetes mellitus.

TABLE :- COMPARISON OF VARIOUS CHARACTERISTICS OF PATIENTS

	MALE		FEMALE		
CHARACTERISTICS	DIABETIC	NON DIABETIC	DIABETIC	NON DIABETIC	P-value
Age (Years) (mean±SD)	52.5± 1.5	54.7 ± 2.1	51.8 ±3.5	55.6 ± 4.2	0.05
Weight (Kg)	69.4±8.9	71.6±13.5	57.7±6.5	62.8±9.4	0.03
BMI (kg/m²)	25.6±3.5	28.9±4.5	27.8±4.2	29.5±4.7	0.03
Coronary Artery disease(Y/No) %	16%	20%	12%	10%	0.04
Systolic Blood Pressure	131.8 ±11.5	137.24±14.8	132.4±11.6	134.8±17.5	0.04
Diastolic Blood Pressure	83.5±14.5	87.6±17	89.7±9.8	84.3±11.2	0.06
Glucose (mg/dL) [Fasting Blood Sugar]	156.4 ±4.5	79.2 ± 4.3	143.5 ± 3.9	87.2 ± 2.1	0.011
Triglyceride (mg/dL)	147.2 ± 4.1	168.4 ± 9.4	152.3 ± 14.1	160.9± 12.8	0.01
Total Cholesterol (mg/dL)	168.3 ± 3.1	182.3 ± 4.9	176.8 ± 9.8	172.4 ± 9.6	0.021
HDL Cholesterol (mg/dL)	32.6 ± 3.1	33.7 ± 3.1	37.9 ± 3.6	37.8 ±4.2	0.031
LDL Cholesterol (mg/dL)	102.4 ± 4.5	104.8 ± 3.9	115.7 ± 12.4	103.4 ± 8.1	0.07
Creatinine (mg/dL)	23.8±11.4	14.8±6.2	41.2±19.8	132.5±5.1	0.06
GlomerularFiltrationRate (ml/min/1.73m²)	59.8±25.1	87.2±24.5	66.8±31.6	91.5±21.5	0.04
Hemoglobin1Ac (%)	8.9±1.2	8.2±2.1	11.2±1.6	12.8±1.1	0.001
NT-proBNP (pg/mL)	524.8±104.2	795.4±208.3	1100.2±312.5	1352.8±354.6	0.003
Troponin T (ng/mL)	1.380±0.9	1.1±0.7	0.9±0.6	1.1±0.6	0.001
OXIDATIVE AND ANTIOXIDATIVE ENZYMES					
Serum peroxides (µmol/L)	82.8±21.4	107.15±26.5	145.6±41.5	162.4±53.9	0.002

SOD (U/g Hb)	895.2±214.5	1278.1±354.6	1198.6±394.5	1078.3±289.4	0.004
Total glutathione (mg/L)	289.75±101.2	312.2±85.4	312.4±154.7	323.6±106.4	0.01
GPx (U/g Hb)	29.5±11.5	31.56±16.4	38.9±10.2	41.5±11.6	0.06
SOD/GPx ratio	34.8±11.5	45.4±13.5	36.8±6.9	26.8±8.5	0.04
ANTIOXIDATIVE MICRONUTRIENTS					
Vitamine C (µmol/L)	18.4±2.1	16.5±1.8	11.4±2.6	15.4±3.1	0.001
ß-carotene (µmol/L)	498.5±124.5	548.45±102.4	478.2±85.7	700.4±254.3	0.02
Zinc (µmol/L)	102.6±40.6	99.8±39.4	152.7±42.4	143.4±32.8	0.005

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