CORRELATION BETWEEN OXIDATIVE STRESS AND TYPE II DIABETIC PATIENTS

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Abstract: Fifty type II diabetic patients (non insulin dependant diabetes mellitus) and 50 healthy individuals (without diabetes) were included in this randomized study. None of the patients studied had any diabetic complications. The levels of serum malondialdehyde (MDA) from type II diabetic patients was 5.33 ± 1.46 nmol/ml as compared to control (2.74 ± 0.4 nmol/ml). There was age-dependent increase in serum MDA in healthy individuals and age-independent increase in MDA levels in type II diabetic patients. There was sufficiently high degree of positive correlation between blood glucose level and the serum MDA level (r=0.78, p<0.001) as well as between the duration of the disease and serum MDA level in TYPE II DIABETIC patients (r=0.82, p < 0.001) indicating that hyperglycemic condition and duration of disease are responsible for increased oxidative stress in these patients.

Key words: Oxidative Stress, Type II Diabetes, Malondialdehyde

INTRODUCTION

Free radicals are continuously produced during aerobic metabolism as a byproduct of mitochondrial electron transport [1-5], during peroxisomal fatty acid metabolism, xenobiotic metabolism and phagocytic attack [6]. Along with this, radiations [7,8], exposure to toxic chemicals and cigarette smoking also release free radicals [9]. In addition advanced glycation endproducts (AGEs) are also very potent source of free radicals [10].

The free radicals, if not scavenged, cause damage to all macromolecules including nucleic acids, carbohydrates, proteins and lipids and the cell organelles, resulting into cellular dysfunctions and cell death. Therefore, cell continuously fights against free radicals with the help of its antioxidant system. Antioxidants are substances that neutralize free radicals or their action [6]. Oxidative defense is provided by enzymatic and nonenzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, chain-breaking scavengers such as vitamin E, vitamin C and glutathione [11-13].

The imbalance between formation of free radicals and the protective antioxidants causes oxidative stress.

There has been considerable debate regarding the extent to which increased oxidative stress contributes towards the development of diabetic complications [14-23]. It has been proposed that oxidative stress may be associated with the pathogenesis of macrovascular complications of type II diabetes [14, 19]. Patients with type II diabetes mellitus showed an increase in lipid peroxidation from the onset of disease [24]. Changes in lipid peroxidation were supposed to be related with underlying metabolic abnormalities in type II diabetes rather than to the onset of complications [25]. Possible sources of elevated free radicals in type II diabetes include increased production of radical oxygen species, especially from glycation or lipoxidation processes, auto-oxidation of glucose and oxidizing of glucose, and decreased antioxidant defense systems [19].

This study was designed i). to determine whether the observed changes in oxidative stress in type II diabetic patients were a manifestation of the illness
or simply a reflection of the ageing process and ii) to check if there is any correlation between MDA and blood glucose levels as well as MDA and duration of disease.

**MATERIALS AND METHODS**

Blood samples were obtained, from 50 patients with type II diabetes of the age 54.46 ± 8.84 years who were attending the fortnightly checkup camp of Diabetic association of Amravati (Regi. No. MH/ 71/3001/Amravati affiliated to Diabetic association of India main branch Mumbai) as well as 50 control subjects of similar age. The selection criteria for the subjects were based on a questionnaire. The questionnaire was intended to elicit information on the subject’s age, duration of diabetes, average blood sugar level from previous reports (minimum five reports), details of caloric intake, body weight, addiction to tobacco chewing, smoking, alcohol consumption etc. occupation, medical usage and any other ailments. Patients under study were without macro and micro vascular complications, chronic renal insufficiency, coronary heart diseases. All the patients were not taking any medicine other than anti-diabetic pills for the past two years. Controls were defined as not having a major medical illness, no hospital admissions, no current medication, and a subjective perception of good health as determined by health questionnaire. The study was approved by the Local Medical Ethical Committee. All the volunteers were informed of the objective of the study and gave their written consent.

**Sample collection:** 3 ml. venous blood from type II diabetic patients and healthy controls (after an overnight fasting) was collected using 5ml disposal syringes into plain bulbs for the measurements of serum malondialdehyde. Samples were placed on ice. The serum was separated within an hour at 3500 rpm, 4°C for 15 min, using cooling centrifuge.

**Glucose measurement:** The fasting blood glucose level was measured using Thyrocare’s Glucometer [26]. 10 -15 μl of blood sample was applied on the test strip and inserted in the insertion window, blood glucose level appeared on electronic display after 60 seconds. This glucose test is based on the measurement of electrical potential caused by the reaction of glucose present in the blood sample with glucose oxidase and potassium ferricyanide present on the test strip. In this reaction electrons are generated, producing a current which is proportional to the glucose in the sample.

**Malondialdehyde measurement:** Malondialdehyde (MDA) was measured by colourimetric estimation of the thiobarbituric acid reacting substance [27]. This test is most widely used for measuring the extent of lipid peroxidation due to its reliability. The basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of TBA form a red coloured malondialdehyde-TBA complex which can be measured colourimetrically at 532 nm.

**Statistical analysis:** The data was analyzed using Karl Pearson’s coefficient of correlation and student t test in MS-Excel.

**RESULTS**

In the control subjects fasting blood glucose level was 88.54 ± 4.7mg/dl, while type II diabetic patients had elevated (178.98 ± 50.42 mg/dl) levels of fasting blood glucose. The difference was highly significant (p<0.001) (Fig. 3).

There was significant (p < 0.001) rise in the level of serum MDA in diabetic patients (5.24 ± 1.3 nM of MDA/ml serum) as compared to controls (2.74 ± 0.4 nM of MDA/ ml of serum). Figure 1 shows a high degree positive correlation between the age of the patients and the MDA levels in nondiabetic controls (r= 0.89) and this coefficient of correlation was highly significant (p < 0.001). In diabetic patients there was only the possibility of positive correlation between the age and the MDA levels (r= 0.39) and the coefficient of correlation was statistically significant (p < 0.0025) this is depicted in figure 2. Figure 3 shows the effect of age on MDA levels in diabetic patients and the control subjects. Figure 4 shows the correlation between levels of blood glucose and MDA levels in diabetic patients. Study showed a significant (p< 0.001) positive correlation (r = 0.79). Study also showed a significant (p < 0.001) positive correlation (r = 0.82) between the duration of diabetes and MDA levels in diabetic patients (Fig. 5).

**DISCUSSION**

The present investigation reveals a high degree of positive correlation between age and the MDA levels in non diabetic control group. Age related increase in
lipid peroxidation have been demonstrated in various animal models as well as in human subjects [28–34]. The increased malondialdehyde levels along with age is due to increased oxidative stress during ageing [1].

As compared to control group, the diabetic patients show a higher level of MDA indicating increased oxidative stress in these patients. This increase in MDA level is not according to the age of the patients. There is a high degree positive correlation between blood glucose level and MDA (r=0.78) as well as duration of diabetes and MDA (r=0.82) in these patients, indicating that in diabetic patients the level of MDA is not dependant on age but on the blood glucose level and the duration of diabetes. The increased oxidative stress in these patients is due to increased blood glucose level. Glucose is a reducing sugar and the aldehyde group of glucose reacts non-enzymatically with free amino groups of proteins, peptides and amino acids to form labile Schiff’s base which subsequently rearranges into stable Amadori product [10, 35–38]. Further, non-oxidative rearrangement and cross linking of Amadori products result in the formation of advanced glycation endproducts (AGEs).
Chemical oxidation and degradation of AGEs result in production of higher number of free radicals. AGEs can bind to their special receptors located on the plasma membrane called as RAGE (receptors for advanced glycation endproducts), which are present on endothelial cells, mononuclear phagocytes, vascular smooth muscle cells and neurons [35-37, 39]. Binding of AGEs to RAGE, stimulates intracellular signal transduction that results in increased oxidative stress [35, 37]. AGEs can activate microglia and other macrophages leading to respiratory burst associated with inflammatory processes such as chemotaxis of mononuclear phagocytes, release of cytokines, interleukins and TNF resulting into cell death as well as increased oxidative stress [40, 41]. Mullarkey et al. [42] have shown that at physiological pH glycated proteins produce fifty fold more free radicals than non glycated proteins. The transition metal catalyzed autoxidation of Amadori products produce superoxide radicals which after dismutation produce hydrogen peroxide. The hydrogen peroxide either enters Fenton type of reaction or reacts with superoxide radical to generate most destructive hydroxyl radicals [43]. These hydroxyl radicals attack on proteins, lipids, carbohydrates as well as DNA causing damage to these macromolecules. When hydroxyl radical attacks on polyunsaturated fatty acids of the membrane, that results into formation of carbon centered lipid radical which on oxidation forms peroxy radical. The peroxy radicals are degraded into malondialdehyde, 4 hydroxy alkenal and 4 hydroxy nonenal [2,44-47], which are the endproducts of oxidative stress. Deshmukh et al. [48] have demonstrated increased oxidative stress and thereby accumulation of lipofuscin granules in the brain of female albino mice due to glycation induced by reducing sugar D galactose.

Thus hyperglycemic condition and duration of disease are responsible for increased oxidative stress in these patients which may be the cause of diabetic complications.

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