INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN SOUTH INDIAN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Article Received on: 18/01/12 Revised on: 21/02/12 Approved for publication: 19/03/12

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ABSTRACT
An imbalance between reactive oxygen species production and antioxidant scavenging has been implicated in type 2 diabetes**.** Reports indicate that several complications of diabetes mellitus are associated with increased activity of free radicals and accumulation of lipid peroxidation products. The aim of this study was to test the hypothesis that type 2 diabetes mellitus is associated with increased oxidative stress in south Indian subjects. Fasting blood samples were obtained from 50 diabetic patients (26 male and 24 female) and 50 (26 male and 24 female) healthy control subjects. Plasma malondialdehyde (MDA), Superoxide dismutase (SOD) reduced glutathione (GSH) and Vitamin C levels were measured and the results were compared with those of controls. MDA levels were found to be significantly higher, SOD and GSH activities were found to be significantly lower in type 2 diabetic patients when compared with controls (P<0.05). There was a decrease in vitamin C level was observed in patients (P<0.05). In this study lower lipid peroxidation is inversely proportional to the antioxidant levels in type 2 diabetics. Therefore, the future studies need to focus on gathering large sample sizes to clarify the relationship between antioxidant depletion and type 2 diabetes mellitus.
KEYWORDS: Oxidative stress; type 2 diabetes mellitus; antioxidants; lipid peroxidation

INTRODUCTION

The prevalence of diabetes is currently estimated to be about 6.4% worldwide and there has been a dramatic increase in the diagnosis of type 2 diabetes in the past two decades[1]. Oxidative stress has been considered to play a central part in the progression of diabetes[2]. Free radicals have important role in the pathogenesis of diabetes and a relationship between oxidative stress and diabetes complications[3, 4].

Excess production of free radicals, mainly due to hyperglycemia, causes oxidative stress, which further aggravates the development of diabetes and its complications[5]. Oxidative stress may be increased in diabetic patients since persistent hyperglycemia causes an increased production of oxygen free radicals through auto oxidation of glucose and non-enzymatic glycation of proteins[6]. Increased levels of the products of oxidative damage to lipids and protein have been detected in the plasma of diabetic patients[7].

In healthy individuals, oxidative damage to tissue is prevented by a system of defenses which includes antioxidant enzymes and vitamins [8]. In diabetic patients an imbalance between reactive oxygen species production and antioxidant levels has been reported[9] but there is still lack of data regarding the actual status of antioxidants in type 2 diabetic patients.

In order to get more information about the activities of enzymatic and non enzymatic antioxidants, in this study levels of TBARS as a marker of lipid peroxidation and SOD, GSH as indices of oxidative stress and Vitamin C as antioxidant vitamins were estimated in patients with type 2 diabetes mellitus and healthy individuals.

MATERIALS AND METHODS

Study Population

The study group consisted of 50 patients with type 2 diabetes mellitus (24 male and 26 female); who were aged 38-55 years. The control group consisted of 50 age and sex matched healthy individuals with normal glucose tolerance tests and the absence of the history of any other diseases. The selection criteria for the subjects were based on a questionnaire. The questionnaire was intended to obtain information on the subject's age, smoking habits, alcohol consumption, duration of disease (type 2 diabetes mellitus), medical usage, and any other diseases. We also ensured that all the subjects had not been taking any medicines other than antidiabetic drugs for the past 3 years. Smoking subjects, individuals taking antiglycemic agents and antioxidant agents were excluded from the study. Informed consent was obtained from each participant before obtaining the blood sample. The procedures were in accordance with the ethical standards of the Helsinki Declaration of 1975.

The fasting venous blood was drawn from diabetic patients and healthy volunteers and immediately transferred to laboratory in an ice box. Each sample was centrifuged at 4000 rpm and the plasma was separated and stored at -20 °C until analysis.

Estimation of Superoxide dismutase (SOD)

Estimation of plasma SOD was done by the method of Kakkar et al[10]. 1.35 ml of double distilled water, 50 µl of plasma, 1.2 ml of sodium pyrophosphate buffer (pH 8.3), 0.1 ml of phenazine methosulphate (PMS) and 0.3 ml of nitroblue tetrazolium (NBT) were mixed. 0.2 ml of NADH solution was added to it to initiate the reaction. After incubation at 39°C for 90 s the reaction was terminated by adding 1 ml of glacial acetic acid. 4 ml of *n* -butanol was added and the mixture was centrifuged at 4000 rpm for 10 min and the absorbance of the upper butanol layer recorded at 560 nm. For the comparison, corresponding blank was prepared in the same way except addition of the plasma. One unit of SOD is defined as the amount of enzyme required for inhibition of the reduction of NBT by 50%.

Estimation of reduced glutathione (GSH)

The GSH level was estimated by the method of Moron *et al* [11]. Briefly, 0.1 ml of plasma was precipitated with 5% TCA and the precipitate was removed by centrifugation. To an aliquot of the supernatant, 2 ml of 5-5'- Dithiobis, 2-

nitrobenzoic acid (DTNB) reagent was added to make the final volume 3 ml. Absorbance was read at 412 nm against a blank containing TCA instead of the sample. The amount of reduced glutathione was expressed as mg/dl of protein.

Estimation of malondialdehyde (MDA)

The TBARS levels were estimated as per the spectrophotometric method described by Ohkawa *et al* [12]. Briefly, to each test tube, 0.5 ml of plasma, 0.5 ml of normal saline, 1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of thiobarbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added. The test tubes were kept for boiling at 95ºC for one hour. To each of the test tubes, 3 ml of n-butanol was added and mixed well. The tubes were centrifuged at 3000 rpm for 10 minutes. The separated butanol layer was collected and read in a spectrophotometer against reagent blank at 535 nm Thiobarbituric reactive substances concentration was expressed in terms of nmol of malondialdehyde per ml of plasma.

Estimation of Ascorbic acid

Plasma ascorbic acid was estimated by dinitrophenyl hydrazine methods with Lowry et al modification [13]. A mixture TCA and plasma (4:1) was centrifuged and mixed with filtered 2.2% dinitrophenyl hydrazine, 5% thiourea, 0.6% copper sulfate (20:1:1) reagent. This mixture was incubated for 1 h in 60°C water bath and immediately chilled on ice-cooled water bath. In this mixture, 65% sulphuric acid was added drop wise, incubated for 25 min at room temperature and absorbance taken at 520nm. A working standard solution of ascorbic acid in acetic acid containing metaphosphoric acid prepared and finally plasma vitamin C concentration was calculated.

Statistical analysis

All data were expressed as mean ± standard deviations (SD). The comparison between two samples was performed by Student's t-test and the *p* values of <0.05 were considered as significant.

RESULTS

The clinical characteristics of the study subjects are shown in Table 1. Mean age of patients and control groups are 41± 8 years and 45.5 ± 6 years respectively. The study groups were well matched for age and sex with their respective control groups.

The mean values of SOD, GSH were significantly ($p<0.05$) decreased while a significant increase in MDA level was observed in type 2 diabetes mellitus in comparison to the respective controls. In addition, the mean value of ascorbic acid was significantly ($p< 0.05$) decreased in the plasma of type 2 diabetics compared to the corresponding values of controls (Table 2).

DISCUSSION

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species which are formed under normal physiological conditions but become deleterious when not being quenched by the antioxidant systems [14]. There are convincing experimental and clinical evidences that the generation of reactive oxygen species is increased in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress [15].

Increased oxidative stress in the pathogenesis of diabetes is not only by rate of oxygen free radical production but also due to nonenzymatic protein glycosylation, auto-oxidation of glucose [16], impaired glutathione metabolism [17], alteration in antioxidant enzymes [18], formation of lipid peroxides [19] and decreased ascorbic acid levels [20]. Unusually high levels of free radicals and simultaneous decline of antioxidant defense systems can lead to the damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of diabetes mellitus[22].

Numerous reports indicate variations in the levels of antioxidants in diabetic patients[23]. In our study the levels of SOD, an enzyme responsible for the scavenging of oxidant stress factors in the body is significantly decreased in type 2 diabetics. Products of membrane lipid peroxidation and other oxidants may react with SOD resulting in oxidative modification thereby causing loss of enzyme activity. GSH is a well-known extracellular antioxidant that protects tissues from the effects of oxidative stress [24]. In our study GSH levels were found to be low in patients. These data suggest that the oxidant/antioxidant balance was altered in favor of oxidants in type 2 diabetics.

Vitamin C is an essential constituent of a healthy diet and also a potent antioxidant that raises cellular defences against oxidative stress. Vitamin C acts as a pro-oxidant in the diabetic state and could be associated with increased free radical formation and lipid peroxidation, which significantly decreased in diabetics [25].

MDA, a lipid peroxidation product, was used as a marker of oxidative stress. An increase in MDA level was observed in our study on type 2 diabetics but not in controls. The association between the parameters which were assessed in this study indicated that the depleted antioxidant status with an increased state of oxidative stress. The other researchers have also reported elevated lipid peroxidation products in blood samples of type 1 and 2 diabetic patients [26, 27].

Thus the increase in lipid peroxides with weakness of the defense antioxidant system in diabetics, probably serves as a background for the pathogenesis of type 2 diabetes. Additional work is needed to confirm whether an association exists in between antioxidant depletion and complications of type 2 diabetes mellitus.

ACKNOWLEDGMENT

The authors are grateful to the Authorities of Karpagam University, Coimbatore, Tamil Nadu, India for the use of facilities and encouragement. We also thank the doctors, nurses and medical assistants of the various clinics in and around Coimbatore, Tamil Nadu, India who helped in sample collection. We thank all the volunteers who took part in this study.

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Table1: Clinical characterization of the study subjects

Parameters	Controls n = 50	Type 2 diabetic subjects n = 50
Sex (M/F)	24/26	24/26
Age (year)	41± 8	45.5 ± 6
Weight (kg)	59.6 ± 6.3	68.5±5.5
Height (cm)	157.0 ± 24.9	159 .6 ± 10.4
Duration of DM (years)	-	12.25 ± 2.4
Family history (%)	52	80

Table 2: Plasma levels of superoxide dismutase (SOD) reduced glutathione (GSH) malondialdehyde (MDA) and Ascorbic acid in type 2 diabetics and healthy controls

Groups	SOD (Unit/ml)	GSH (mg/dl)	MDA (nmol/l)	Ascorbic acid (mg/dl)
Control	10.1±1.75*	4.39±0.58*	3.21±0.22	6.16±0.19*
Type 2 diabetics	5.3±0.86	3.04±0.38	7.12 ±0.45*	3.12±0.03

All data are shown as mean ± SD; *p<0.05 significant vs. control subjects.

Source of support: Nil, Conflict of interest: None Declared