Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients

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ABSTRACT

Introduction: The depletion of defensive body chemicals called antioxidants may increase the risk of complications from the most common form of diabetes mellitus. This study aims to evaluate blood serum lipid peroxidation product (malondialdehyde), an antioxidant, in non-insulin dependent male and female type 2 diabetic patients.

Methods: Blood serum samples were collected from the diabetic patients and non-diabetic healthy controls. Malondialdehyde (MDA) level, which is an index of endogenous lipid peroxidation, reduced glutathione and protein content of the serum were estimated.

Results: A significant elevation in MDA level and decrease in glutathione and protein content was observed in both male and female diabetic patients in comparison to non-diabetic controls.

Conclusion: Our findings indicate that the increase in the lipid peroxidation product MDA and decline in glutathione-dependent antioxidant defences may appear early in non-insulin dependent type 2 diabetes mellitus patients before the development of secondary complications.

Keywords: antioxidant, diabetes mellitus, malondialdehyde, non-insulin dependent type 2 diabetes mellitus, oxidative stress

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INTRODUCTION

Diabetes mellitus is characterised by hyperglycaemia together with biochemical alterations of glucose and lipid peroxidation[1]. Some complications of diabetes mellitus are associated with increased activity of free radical-induced lipid peroxidation and accumulation of lipid peroxidation products[2]. 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. It has been found to be connected with various disease processes, such as carcinogenesis, atherosclerosis and hypertension[3]. It is also involved in oxidative stress, which plays a major role in the pathogenesis of diabetic mellitus[4]. To control lipid peroxidation, there is a defensive system consisting of antioxidant enzymes that play an important role in scavenging reactive oxygen species[3]. 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 defences.

Abnormally high levels of lipid peroxidation and the simultaneous decline of antioxidant defence mechanisms can lead to damage of cellular organelles and lead to oxidative stress. Many reports were available with regard to oxidative stress and antioxidant status of type 2 diabetic patients[5-7]. However, studies carried out in India in type 2 diabetic patients on the status of lipid peroxidation and glutathione levels are scarce[8]. In the present study, the relationship between level of serum lipid peroxidation product, malondialdehyde (MDA), an oxidant, reduced glutathione level, an antioxidant and protein content in male and female non-insulin dependent type 2 diabetic mellitus (NIDDM) patients was investigated against healthy non-diabetic volunteers as controls.

METHODS

This study was conducted on 70 cases (44 male and 26 female) of NIDDM patients, who were attending our health centre for their monthly routine medical examination, and 59 healthy (33 male and 26 female) controls with similar age and sex distribution. The selection criteria for the subjects were based on a questionnaire. The questionnaire was intended to elicit information on the subject's age, smoking habits, alcohol consumption, duration of disease (type 2 diabetes mellitus), medical usage, and any other ailments. We also ensured that all the subjects had not been taking any medicines other than antidiabetic pills for the past 3-5 years. The patients in the study had normal hepatic and endocrine functions and were relatively well controlled with

Table I. Clinical characteristics of type 2 diabetic patients and controls.

	Male		Female	
	Diabetic (n=44)	Controls (n=33)	Diabetic (n=26)	Controls (n=26)
Mean age (in years)	51.5 ± 1.5	49.5 ± 2.2	54.4 ± 2.6	53.5 ± 2.3
Diabetic duration (in years)	4.0 ± 0.3	–	4.0 ± 0.3	–
Glucose level (mg/dL)	149.2 ± 3.8*	76.2 ± 3.8	136.7 ± 9.8*	82.5 ± 1.7
Triglyceride level (mg/dL)	142.2 ± 3.7	178.0 ± 3.8	139.5 ± 13.7	160.4 ± 18.6
Total cholesterol (mg/dL)	169.3 ± 3.8	176.9 ± 3.7	174.6 ± 7.8	171.5 ± 7.6
HDL cholesterol (mg/dL)	31.6 ± 3.7	32.4 ± 3.8	36.6 ± 2.6	36.8 ± 2.4
LDL cholesterol (mg/dL)	99.7 ± 3.7	102.7 ± 3.8	112.6 ± 10.2	100.9 ± 7.0
VLDL cholesterol (mg/dL)	28.6 ± 3.7	17.4 ± 3.9	33.3 ± 3.7	28.7 ± 2.8

Values are given as mean ± standard error, rounded to one decimal place. Each experiment repeated three times.

*significantly different from control, $p<0.05$

Table II. MDA, glutathione and protein levels in blood serum of type 2 diabetic patients and controls.

	Male		Female	
	Diabetic	Controls	Diabetic	Controls
MDA level (pmoles MDA formed/mg protein)	0.29 ± 0.04*	0.11 ± 0.02	0.23 ± 0.02*	0.07 ± 0.01
Glutathione level (pmoles/mg protein)	195.6 ± 11.4*	294.8 ± 12.1	193.9 ± 10.9*	250.3 ± 11.9
Protein level (mg/ml)	86.1 ± 0.4*	104.4 ± 0.5	95.0 ± 0.7	101.5 ± 0.5

Values are given as mean ± standard error. Each experiment repeated three times.

*significantly different from control, $p<0.05$

glycated haemoglobin (HbA1c) <6-7% (normal range <8%).

Patients with macro- and microangiopathic complication, coronary heart disease or hypertension, chronic renal insufficiency, uncontrolled primary/secondary hypertension, and other life-threatening diseases, such as cancer, were excluded from the study. All the patients were taking oral antidiabetic drugs at the time of study. The recruited subjects were informed of the objective of the study and gave their consent. Before enrolling in the study, all subjects were screened for medical history, physical examination, electrocardiography (ECG), fasting blood sugar, total cholesterol, HDL/LDL cholesterol, triglycerides and these parameters were within the reference intervals (Table I). The LDL cholesterol was measured directly. Laboratory measurements used routine diagnostic reagents from Bayer Diagnostics India Ltd. To avoid possible bias, the samples were coded. The institutional ethical committee approved the research procedures used in this study.

The fasting venous blood was drawn from diabetic patients and healthy volunteers around 900 am and the serum was separated immediately by centrifugation at 1000g for 15 min using cooling research centrifuge (REMI, model CPR-24). The protein content in serum of diabetic and non-diabetic patients was estimated as described by Lowry et al[9]. Lipid peroxidation product, MDA, was measured by the method as outlined by Wills[10]. The reduced glutathione level was measured by adopting the method described by Ellman[11]. These biochemical parameters were determined spectrophotometrically using double beam spectrophotometer (UV-2101, Shimadzu, Japan). All values were expressed as the mean obtained from the number of experiments (n). Data from all the tables of normal and diabetic patients were compared by ANOVA followed by Student's t-test as described by Mahboob et al[12]. P-values of less than 0.05 were considered to be significant.

RESULTS

The male and female type 2 diabetic patients had significantly elevated levels of fasting blood glucose (Table I), whereas the control subjects consisting of both male and female human volunteers had normal blood glucose level and without diabetic complications. The study groups were well matched for age and sex with their respective control groups. There were no significant differences in triglycerides,

total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol in diabetic male/female patients, compared to controls (Table I). The serum MDA level, a product of lipid peroxidation, increased significantly, whereas the reduced glutathione and protein content decreased significantly in male and female diabetic patients in comparison to non-diabetic volunteers (Table II).

DISCUSSION

Increasing evidence in both experimental and clinical studies suggest that oxidative stress plays a major role in the pathogenesis of both type 1 and type 2 diabetes mellitus. Free radicals are formed disproportionately in diabetes mellitus by glucose degradation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation, which may play an important role in the development of complications in diabetic patients. The generation of free radicals may lead to lipid peroxidation and formation in several damage in diabetes mellitus. In the present study, we have observed that MDA levels, a lipid peroxidation product and a marker of oxidative stress, were elevated significantly in male and as well as in female diabetic patients (Table II). This clearly shows that 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 1 and 2 diabetic patients[4,13-15].

Abnormally-high levels of free radicals, lipid peroxidation and simultaneous decline in antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes. These consequences of oxidative stress can promote the development of complications in diabetes mellitus patients. Antioxidant enzyme-dependent defences play an important role in scavenging free radicals produced under oxidative stress[16]. Our data reveal that glutathione, an antioxidant, and the protein content of blood serum of diabetic mellitus patients were significantly low (Table II), indicating decreased scavenging capacity of glutathione-dependent anti-oxidant defensive system against elevated lipid peroxidation processes in these patients.

Sailaja et al[8] reported that diabetic humans have 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[17]. The diabetic adult rats have also shown an increase in MDA level, whereas antioxidant enzymes such as reduced glutathione, superoxide dismutase and glutathione peroxidase activities were markedly diminished in comparison to controls[18,19]. The results suggest that the increase in lipid peroxidation and the decline in antioxidant defences may appear early in type 2 non-insulin dependent diabetes mellitus patients, before the development of secondary complications.

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