

Lipid Peroxidation and Resistance to Oxidation in Patients with Type 2 Diabetes Mellitus

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PAŞAOĞLU, H., SANCAK, B. and BUKAN, N. *Lipid Peroxidation and Resistance to Oxidation in Patients with Type 2 Diabetes Mellitus*. Tohoku J. Exp. Med., 2004, **203** (3), 211-218 — We investigated lipid peroxidation, resistance of plasma and red blood cells to oxidation, and antioxidant defense system in erythrocytes and sera in patients with type 2 diabetes mellitus. One group included newly diagnosed 20 patients and the other included 20 patients treated with oral antidiabetic agents (OAD). Twenty healthy subjects served as controls. Serum and red blood cell malondialdehyde (MDA), glutathione (GSH), resistance to oxidation, and plasma thiol (total -SH) levels were measured. In addition, glycated hemoglobin, serum fructosamine, uric acid, total protein, total cholesterol, triglyceride and glucose levels were determined. Although newly diagnosed patients had higher serum and erythrocyte MDA levels than those of controls, the highest levels of MDA were determined in patients treated with OAD. MDA levels after exposing to oxidation increased in OAD group more than in newly diagnosed patients. Total -SH and erythrocyte GSH levels of the both diabetic groups were lower than controls. These results show that serum and erythrocyte lipid peroxidation was increased in diabetic patients. The sera of the patients showed a decreased resistance against oxidation. We therefore suggest that the effect of increased free radicals may be prevented by antioxidant systems in early stages of type 2 diabetes but in advanced stages this relationship is impaired owing to decreased antioxidant activity. Decreased red blood cell GSH and serum total -SH levels may be due to a compensation mechanism of the antioxidants. — diabetes mellitus; lipid peroxidation; antioxidants
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Diabetes is recognized as one of the leading causes of morbidity and mortality in the world. Type 2 diabetes occurs predominantly in adults over than 30 years old. About 2.5-3% of the world's population suffers from this disease (American Diabetes Association 1995). Also in

our country can reach 6.9% (Kelestimur et al. 1999). Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation (Giugliano et al. 1996). An increased oxidative stress has been observed in diabetic patients as indicated by high free radical

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production (Seghrouchni et al. 2002). The source of free radicals in diabetes is not understood in detail, but glycation of proteins can lead to oxidative stress by direct release of O_2^- and H_2O_2 and activation of phagocytes through a specialized receptor for advanced glycation end products (Abuja and Albertini 2001). To counter the effect of free radicals, the body is endowed with another category of compounds called antioxidants. These antioxidants are produced either endogenously or received from exogenous sources and include enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase, minerals like Se, Mn, Cu and Zn, and vitamins like vitamin A, C and E. Other compounds with antioxidant activity include glutathione, flavonoids, bilirubin and uric acid (Irshad and Chaudhuri 2002). Antioxidants such as reduced glutathione (GSH), uric acid are supposed to protect, can prevent or reduce the extent of oxidative destruction of biomolecules (Halliwell 1990).

Long-term complications of type 2 diabetes include retinopathy, nephropathy, atherosclerosis (Konukoglu et al. 1999; Zelko et al. 2002), and shortened life span of erythrocytes (Lahrman 1977; Jain et al. 1989). Increased free radical production is said to mediate tissue injury in a wide range of diseases and diabetes mellitus is no exception (Yildiz et al. 2002). Diabetes-induced oxidative damage may be more prominent in red cells compared to other tissues due to their high content of iron and polyunsaturated fatty acids, their role as an oxygen transporter, and due to their protection of the host in vivo by neutralizing exogenous and endogenous free radicals behaving as expendable scavengers competing with other tissues for the toxic effects of free radicals (Richards et al. 1998).

The present study was designed to evaluate lipid peroxidation end product such as serum and erythrocyte malondialdehyde (MDA) levels and resistance to oxidation of newly diagnosed patients (group I), and the patients treated with oral antidiabetic agents (OAD) (group II) and the healthy normal subjects. In addition to this

we measured antioxidants such as total -SH, uric acid, and glutathione levels in all groups. The measurement of plasma total thiol (-SH) is a good reflection of excess free radical generation, since the conformation of albumin is altered, allowing -SH groups to be oxidized (Hu 1994; Bourdon et al. 1999).

MATERIALS AND METHODS

Subjects

This study was planned with the approval of the local ethic committee and included 60 individuals: 20 patients with newly diagnosed type 2 diabetic patients, 20 type 2 diabetic patients treated with OAD and 20 healthy control subjects. The clinical and biochemical characteristics of diabetic patients and their age-matched controls are summarized in Table 1. None of the patients was known to suffer from an acute illness or chronic inflammatory condition at the time of the study. Blood samples were drawn in the fasting state and processed within 1 hour of collection. The samples were obtained from antecubital vein using a 19 gauge sterile needle and blood was allowed to flow freely into vacutainer tubes containing EDTA (to analyze erythrocyte MDA, GSH) or no additive (to analyze serum parameters). All samples were taken in the morning to avoid the confounding effect of diurnal variation of oxidative stress parameters as reported previously (Bridges et al. 1992).

For erythrocyte MDA, glutathione and haemoglobin analysis, erythrocytes were separated from plasma (EDTA was used) and prepared erythrocyte lysate by washing four times with saline. These lysates were stored at -70°C until analysis. All other parameters were determined on the same day of collection.

Glycated Hemoglobin (HbA1C) (%)

For this analysis, Shimadzu SPD-10A UV-Vis detector (Shimadzu, Kyoto) and Chromsystems HPLC kits were used. Results were given as the percentage of all resolved hemoglobin subfractions.

TABLE 1. *Clinical and chemical characteristics in diabetic and control groups*

	Group I (Newly diagnosed)	Group II (OAD)	Group III (Control)
<i>n</i>	20	20	20
Female/male	11/9	12/8	12/8
Age (years)	48.4±9.5	53.0±8.8	47.2±9.9
Duration of DM (years)	-	7±3	-
Glucose (mg/100 ml)	198±73 ^a	185±73 ^a	90±12
HbA1C (%)	8.9±2.7 ^a	9.3±2.7 ^a	5.4±0.6
Fructosamine (μmol/liter)	756±187 ^a	1127±272 ^{a,b}	170.25±49.16
Total protein (g/100 ml)	7.3±0.4	7.3±0.3	7.1±0.5
Uric acid (mg/100 ml)	4.4±1.3	4.0±0.8	4.6±1.2
T. cholesterol (mg/100 ml)	236±52 ^a	203±26 ^b	185±40
Triglycerides (mg/100 ml)	215±102 ^a	166±82 ^b	145±67

^a*p*< 0.01 significant compared to group III, ^b*p*< 0.01 significant compared to group I.

Fructosamine level (μmol/liter)

Fructosamine was determined in serum with the colorimetric assay which based on the ability of ketoamines to reduce nitrobluetetrazolium (NBT) to formazan in alkaline solution using Abbott-Aeroset autoanalyzer (Chicago, IL, USA).

Biochemical parameters

Glucose (mg/100 ml), total protein (g/100 ml), uric acid (mg/100 ml), total cholesterol (mg/100 ml), triglycerides (mg/100 ml) levels were measured with original kits using Abbott-Aeroset autoanalyzer (Chicago, IL, USA).

Oxidative stress markers

Malondialdehyde levels (μmol/liter). Serum MDA levels were determined as described by Satoh (1978) by spectrophotometry at 532 nm after boiling the sample and condensing it with thiobarbituric acid (TBA). The coefficient of variability between assays was 5.1%.

Resistance to oxidation

Resistance of serum samples of patients and healthy controls were measured after addition of 50 μl coppersulfate (10 μmol/liter) and 50 μl hydrogenperoxide (300 ml/liter) to 1 ml serum. The mixture were incubated at 37°C water bath.

Then resistance autooxidation of serum samples were determined using the method of Stocks et al. (1974). By determination of the negative correlation between the degree of production of lipid peroxidation end-products and the resistance of oxidation, the total antioxidant status of the patients and control subjects were evaluated.

Erythrocyte MDA levels

MDA concentrations of erythrocytes were analyzed, as described by Ohkawa et al. (1979). Erythrocyte haemoglobin levels were also measured by cyanomethaemoglobin method (Fairbanks and Klee 1994). The results were expressed as nmol MDA/g Hb.

Total -SH concentration

Total -SH concentration was determined by using 5-5'-dithio-bis (2-nitrobenzoic acid) (DTNB) as described by Hu (1994). Absorbance were measured at 412 nm against blank samples without DTNB.

Erythrocyte reduced glutathione levels (μmol/g Hb)

Erythrocyte GSH levels were determined in whole blood specimens from EDTA containing tubes, according to the method of Beutler et al.

(1963).

Statistical analysis

All results are expressed as mean \pm S.D. A Student's *t*-test was used to estimate differences between the groups. The criterion for significance was $p<0.05$.

RESULTS

Clinical and biological data of healthy and diabetic subjects are summarized in Table 1 and oxidative stress markers in Table 2.

Diabetic patients present an abnormal lipid profile with significant higher serum level of total cholesterol and triglyceride compared to healthy control subjects ($p<0.01$, in both). In addition to this, total cholesterol and triglycerides were significantly different between diabetic subjects (groups I and II). Group I had higher total cholesterol and triglycerides levels than group II ($p<0.01$).

Total protein and uric acid levels were normal in all groups. These parameters were not significantly different between the groups.

HbA1C, serum glucose and fructosamine levels were increased in group I and group II when compared with the healthy subjects ($p<0.01$, in all). Although glucose and HbA1C levels did not differ between the diabetic groups (groups I and II), fructosamine level was significantly higher in group II than those in group I ($p<0.01$).

Serum MDA levels were significantly higher in group I and group II compared to controls

($p<0.05$). Similar to this, erythrocyte MDA levels were significantly higher in group I and group II compared to controls ($p<0.05$). Although erythrocyte MDA levels were significantly enhanced in group II than group I ($p<0.05$), serum MDA levels were not different between groups I and II.

Control group had significantly higher values of erythrocyte reduced glutathione compared to group I and group II ($p<0.05$).

Sulphydryl groups (total -SH) were significantly lower in diabetic patients (both groups I and II) compared to controls ($p<0.05$).

DISCUSSION

Free radicals are considered to have a great role in the pathogenesis of many diseases such as diabetes mellitus, atherosclerosis, cell damage, cancer, myocardial infarct, haemolytic and immune disease. The levels of these reactive oxygen species are controlled by antioxidant enzymes namely, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and nonenzymatic scavengers like reduced GSH, vitamin E, selenium, β -carotene, coenzyme Q. The reports indicate increased or decreased lipid peroxidation, SOD, GSH-Px and CAT activity in various tissues; like liver, kidney, blood vessels, heart, lymphoid organs, lungs and uterus (Ramanathan et al. 1999; Kinalski et al. 2000).

Merzouk et al. (2003) investigated plasma vitamin A, C and E levels and erythrocyte antioxidant enzyme activities in type I and type II diabetic subjects with and without complications.

TABLE 2. Oxidant and antioxidant levels of patients.

	Group I (Newly diagnosed)	Group II (OAD)	Group III (Control)
Serum MDA (μ mol/liter)	2.18 \pm 0.47 ^a	2.52 \pm 0.51 ^a	1.52 \pm 0.51
MDA after oxidation (μ mol/liter)	3.00 \pm 1.38 ^a	4.7 \pm 2.44 ^{a,b}	2.02 \pm 1.36
Total -SH (μ mol/liter)	605.64 \pm 36.02 ^a	604.61 \pm 56.06 ^a	664.79 \pm 60.13
Uric acid (mg/100 ml)	4.4 \pm 1.3	4.0 \pm 0.8	4.6 \pm 1.2
Erythrocyte MDA (nmol/g Hb)	756 \pm 187 ^a	1127 \pm 272 ^{a,b}	707 \pm 266
Erythrocyte GSH (μ mol/g Hb)	7.55 \pm 2.12 ^a	7.81 \pm 1.18 ^a	8.77 \pm 1.03

^a $p<0.05$ significant compared to group III, ^b $p<0.05$ significant compared to group I.

However, vitamin A and E levels were significantly lower in type I and type II diabetic subjects compared to controls, similar with other studies (Hasanain and Mooradian 2002; Ahmad et al. 2003). In addition to this plasma SOD and CAT activities were found lower in type 2 diabetic group than control group.

Malondialdehyde is one of the lipid peroxidation products frequently used to determine the oxidant/antioxidant balance in diabetic patients (Altomare et al. 1992; Gallou et al. 1993). However, there are contradictory results in literature about serum MDA levels in patients with diabetes. Sundaram et al. (1996) studied 467 cases of type 2 diabetics and found that lipid peroxidation was significantly raised in plasma and erythrocytes. In our study, we found significantly elevated MDA levels in serum and erythrocyte of diabetic patients.

Higher levels of HbA1C and fructosamine in patients with diabetes is thought that they have poor glycemic control. In addition to this, patients treated with oral antidiabetic agents (group II) had higher MDA levels than newly diagnosed patients (group I).

On the other hand Bates et al. (1997) found normal MDA level in diabetic patients. High levels of glucose can produce permanent chemical alterations in proteins and increase lipid peroxidation in a variety of experimental models of hyperglycemia (Wolff and Dean 1987; Jain and Lim 2001). Hyperglycemia, itself, may stimulate platelet aggregation (Dousset et al. 1983; Wohaieb and Godin 1987) and autooxidation of glucose may also lead to free radical production in diabetics (Hunt et al. 1988).

Plasma thiols and uric acid (Hu 1994; Elhadd et al. 1998; Bourdon et al. 1999) and red cell GSH (Collier et al. 1990; Dominguez et al. 1998; Elhadd et al. 1998) are physiological free radical scavengers. Protein thiols may serve an antioxidant function by several mechanisms. Protein thiols may preemptively scavenge oxidants which initiate peroxidation. Telci et al. (2000) found that plasma thiols was significantly decreased between

the diabetic groups than their age-matched controls. Wittenstein et al. (2002) also found lower plasma thiol levels in type I diabetes compared to the controls. Our findings are in concordance with their results.

A potential beneficial effect of elevated uric acid levels generating an increased antioxidative defense in adults has been discussed (Wittenstein et al. 2002). In humans, uric acid is the main plasma antioxidant followed by vitamin C. Uric acid stabilizes vitamin C in plasma and protects it from oxidation (Ames et al. 1981).

GSH may be a primary agent involved in redox regulation of protein thiols (Packer et al. 1995). There is a consensus that reduced/oxidized glutathione ratio is lower in diabetes (Collier et al. 1990; Baynes 1991; Dominguez et al. 1998; Elhadd et al. 1998; Cakatay et al. 2000). In hyperglycemic conditions, glucose is preferentially used in the polyol pathway (Lee and Chung 1999), that consumes NADPH which is necessary for GSH regeneration by the glutathione reductase enzyme. Hyperglycemia is therefore indirectly the cause of GSH depletion. As GSH is an important molecule, its depletion leads to the increase of oxidative stress.

As the key intracellular antioxidant, GSH reacts with electrophilic compounds and serves as a reductant for eliminating hydrogen peroxides (Bannai and Tateishi 1986). The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the structure and permeability of membrane. We have shown that red blood cell GSH levels decreased in our diabetic patients parallel to the increase of MDA. However, there was not a significant difference between the diabetic groups. Our results are consistent with those of other studies on antioxidant status of diabetic patients (Collier et al. 1990; Altomare et al. 1992; Gallou et al. 1993). Antioxidant molecule, GSH, significantly decreased as shown in other studies (Giugliano et al. 1996; Nourooz-Zadeh et al. 1997). Sundaram et al. (1996) confirm the relationship between the period of illness and GSH depletion. In long term

hyperglycemic conditions, GSH depletion was more significant than those of newly diagnosed patients.

In the present study, sera from patients using OAD agents were found to be more susceptible to oxidation than those of newly diagnosed subjects. Konukoglu et al. (1999) verified that the type 2 diabetic patients with or without angiopathy had higher lipid peroxide levels than those of control. But when lipid peroxides treated with hydrogen peroxides MDA levels became higher in diabetic patients with angiopathy as compared to diabetic patients without angiopathy.

Some differences between previous studies and our investigation may be related to the regulation of disease or the duration of diabetic condition.

In conclusion, we showed that newly diagnosed patients and patients using OAD had elevated peroxidation and decreased antioxidant activity.

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