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Urinary Tract Infection Aggravates Oxidative Stress in Diabetic Patients

MUSTAFA GUL, ERGUL KURUTAS,[1] PINAR CIRAGIL, ALI CETINKAYA,[2] METIN KILINC,[1] MURAT ARAL and MEHMET AKIF BUYUKBESE[2]

Department of Clinical Microbiology, [1]Department of Biochemistry, and [2]Department of Internal Medicine, Kahramanmaras Sutcu Imam University, Faculty of Medicine, Kahramanmaras, Turkey

GUL, M., KURUTAS, E., CIRAGIL, P., CETINKAYA, A., KILINC, M., ARAL, M. and BUYUKBESE, M.A. *Urinary Tract Infection Aggravates Oxidative Stress in Diabetic Patients*. Tohoku J. Exp. Med., 2005, **206** (1), 1-6 —— To investigate the effect of urinary tract infection on oxidative stress in diabetic patients, we measured the activities of antioxidant enzymes such as catalase and superoxide dismutase, and lipid peroxidation levels in urine specimens of type II diabetic patents with urinary tract infection. A total of 69 patients were included into this study: 23 non-diabetic patients with urinary tract infection, 28 patients with diabetes mellitus, and 18 diabetic patients with urinary tract infection. Twenty-five healthy subjects, matched for age, sex, body mass index and smoking status were also included as control. Urine cultures were performed by the standard techniques, and all grown bacteria were identified as *Escherichia coli*. Antioxidant enzymes and lipid peroxidation levels in urine were measured by spectrophotometric method. In urine samples of diabetic patients with or without urinary tract infection and in urine samples of non-diabetic patients with urinary tract infection, catalase and superoxide dismutase activities were lower and lipid peroxidation levels were higher than those of the healthy subjects ($p < 0.05$). Diabetic patients without urinary tract infection were similar to non-diabetic patients with urinary tract infection. Decreased antioxidant capacity and the increased levels of lipid peroxidation were profoundly higher in diabetic patients with urinary tract infection. These results indicate that urinary tract infection aggravates the oxidative stress in diabetic patients. Therefore we believe that diabetic patients with urinary tract infection need antioxidant treatment. ———— urinary tract infection; diabetes mellitus; antioxidant enzymes; lipid peroxidation; *Escherichia coli*

Cellular oxidative damage is a well-established general mechanism for cell and tissue injury and primarily caused by reactive oxygen species (ROS). These ROS can bind with most normal cellular components; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids (Floyd 1990; Trush and Kensler 1991; Stevens and Nerishi 1992; Uotila et al. 1994). A disturbance of the balance between formation of active oxygen metabolites and the rate at which they are scavenged by enzymatic and nonenzymatic antioxidants is

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Correspondence: Mustafa Gul, M.D., Department of Clinical Microbiology, Faculty of Medicine, Kahramanmaras Sutcuimam University, Yoruk Selim Mah. Hastane Cad. No. 32, 46050, Kahramanmaras, Turkey.
e-mail: mustafagultr@yahoo.com

referred to as oxidative stress (Papas 1996). It has been suggested that oxidative stress plays an important role in some physiological conditions and in many diseases, including diabetes mellitus (DM), myocardial infarction and carcinogenesis. Cells and biological fluids have an array of protective antioxidant mechanisms such as glucose-6-phosphate dehydrogenase, superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase and reduced glutathione, for both preventing the production of free radicals and repairing oxidative damage (Chandra et al. 1994).

Urinary Tract Infection (UTI) is common in all age groups, and may range clinically from asymptomatic bacteriuria to urosepsis (Sobel and Kaye 2000). A variety of oxidation products are found in urine and thought to mirror local and systemic oxidative stress (Kirschbaum 2001). Urinary malondialdehyde (MDA) is found in increased quantities under conditions such as alpha thallassemia, renal failure and pancreatic diseases (Sumboonnanonda et al. 1998; Kang et al. 2001; Okur et al. 2003). Recently a number of investigators have studied ROS and the antioxidant systems or MDA in serum, erythrocytes or plasma of diabetic patients (Duman et al. 2003; Opara 2004; Paşaoğlu et al. 2004; Peuchant et al. 2004; Santilli et al. 2004). But the changes occuring in the urine of diabetic patients are obscure and according to our knowledge, no report is available regarding the MDA levels and antioxidant enzymes in urine of diabetic patients with UTI. The present study aimed to determine the effect of UTI on antioxidant systems and MDA levels in the urine samples of patients with type II DM.

Materials and Methods

Patients and urine samples

A new criteria presented in 1995 by the American Diabetes Association was taken as diagnostic criteria for DM (DeFronzo 1998). The informed consent from all subjects and the local ethics committee approval were obtained. Eighteen patients were diagnosed with DM associated with UTI. Twenty-eight diabetic patients without UTI were collected from the Department of Internal Medicine. The 23 non-diabetic patients with UTI and twenty-five age, sex, body mass index and smoking status matched healthy persons were also included into the study. The characteristics of subjects are given in Table 1. Midstream urine samples were obtained using clean-catch technique and transmitted to the Microbiology Laboratory in about half an hour. All samples were inoculated into the Mac Conkey and blood agar and incubated at 37ºC for 18 - 24 hours. Bacteria 10^5 cfu/ml or over in blood agar were accepted as the cause for UTI. Urinary isolates from positive cultures were identified by using automated bacteria identification systems (API ID 32E, Biomérieux, France). The urine samples were prepared with diluted 50-fold in 0.9% NaCl and stored at

Table 1. *The characteristics of patients and healthy subjects*

	Sex		Age	Body mass	Smoking status	
	Male	Female			Yes	No
Diabetic patients with UTI (n = 18)	8	10	43 ± 3	76 ± 7	-	18
Diabetic patients without UTI (n = 28)	12	16	45 ± 4	70 ± 8	2	26
Non-diabetic patients with UTI (n = 23)	10	13	39 ± 5	67 ± 8	1	22
Healthy subjects* (n = 25)	11	14	41 ± 8	74 ± 5	1	24

UTI, urinary tract infection.

*Healthy subjects and patients were similar for sex, age, body mass and smoking status (p < 0.05).

- 20ºC until the assay.

Biochemical analysis

SOD activity was measured according to the method described by Fridovich (1983). This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with p-iodonitrotetrazolium violet to form a red formazon dye which was measured at 505 nm. Assay medium consisted of the 0.01 M phosphate buffer and CAPS (3-cyclohexilamino-1-propanesulfonicacid) buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with a pH of 10.2. SOD activity was expressed as U/mg protein.

CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler (1975). The assay medium consisted of 1 M Tris HCI, 5 mM Na_2EDTA buffer solution (pH 8.0), 1 M phosphate buffer solution (pH 7.0), and 10 mM H_2O_2. CAT activity in urine was expressed U/mg protein.

MDA levels in the urine samples were measured according to procedure of Ohkawa et al. (1979). The reaction mixture contained 0.1 ml sample, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid. The mixture pH was adjusted to 3.5 and volume was finally made up to 4.0 ml with distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1, v/v) were added. The mixture was shaken vigorously. After centrifugation at 4,000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. MDA level in urine was expressed nmol/mg protein.

The protein concentration of the urine samples was measured with Shimadzu-UV 1601 (Shimadzu, Tokyo) spectrophotometer by the method of Lowry (1951).

HbA1c (glycosylated hemoglobin) levels were determined by using High Performance Liquid Chromatography with Drew Scientific (Cumbria, England). The serum glucose levels were measured on a Dade Behring RAX (Liederbach, Germany) automated analyzer using heksokinase.

Statistical analysis

Statistical analysis was carried out with the SPSS - X (Release 4.1) program. All data are expressed as the mean ± standard deviation. The patients and healthy persons for each parameter were compared with Mann-Whithney's U-test. Differences are considered significant when the probability is less than $p < 0.05$.

Results

There was no difference with respect to age, sex, smoking status and body mass index among the groups. Pathogen bacteria were grown in all patients with urinary tract infection (41 patients). All grown bacteria were identified as *Escherichia coli* (*E.coli*). No other strains grew in the culture of all specimens.

The results of glucose and HbA1c levels are given in Table 2. Glucose and HbA1c levels were elevated in the Type II diabetics as compared to non–diabetic patients with UTI and healthy subjects. There were statistical differences in serum glucose levels and HbA1c levels between them ($p < 0.05$).

As shown in Table 3, diabetic patients with UTI showed higher oxidation levels in urine com-

Table 2. *The levels of HbA_{1c} and glucose in patients and healthy subjects*

	n	Glucose (mg/100 ml)	% HbA_{1c}
Diabetic patients with UTI*	18	139.16 (± 14.11)	9.92 (± 0.23)
Diabetic patients without UTI*	28	138.67 (± 12.31)	9.77 (± 0.48)
Non-diabetic patients with UTI	23	88.47 (± 2.18)	4.81 (± 0.26)
Healthy subjects	25	87.81 (± 2.26)	4.65 (± 0.37)

UTI, urinary tract infection.

*Significant differences in serum glucose levels and HbA1c levels in the Type II diabetic patients compared to healthy subjects and patients with UTI ($p < 0.05$).

TABLE 3. *The levels of malondialdehyde and the activities of catalase and superoxide dismutase enzymes in urine specimens*

	CAT (U/mg protein)	SOD (U/mg protein)	MDA (nmol/mg protein)
Diabetic patients with UTI	0.39 ± 0.10	2.50 ± 0.99	1.84 ± 0.65
Diabetic patients without UTI	0.57 ± 0.16	4.16 ± 1.85	0.59 ± 0.22
Non-diabetic patients with UTI	0.68 ± 0.18	5.64 ± 1.60	0.78 ± 0.30
Healthy subjects*	0.81 ± 0.45	8.69 ± 3.59	0.22 ± 0.10

CAT, catalase; SOD, superoxide dismutase; MDA, malondialdehyde.

*Significant differences in CAT, and SOD activities and MDA levels between healthy subjects and patients ($p < 0.05$).

pared to diabetic patients without UTI and non-diabetic patients with UTI ($p < 0.05$). The antioxidant enzyme activities were lower and lipid peroxidation levels were higher in urine samples from diabetic patients with and without UTI and non-diabetic patients with UTI compared to that from healthy subjects ($p < 0.05$). The antioxidant enzyme activities were lower and MDA levels were strikingly higher in urine samples from diabetic patients with UTI compared to that from healthy subjects ($p < 0.05$). However, there was no significant difference between diabetic patients without UTI and non–diabetic patients with UTI ($p > 0.05$).

DISCUSSION

In this study we used age, sex, body mass index and smoking status matched group. By that we discarded the parameters which may interfere with the oxidative status of the body.

DM is not simply a disorder of glucose homeostasis but also accompanied by various degenerative manifestations (Betteridge 1989) which are related to the overproduction of ROS and dysfunction of antioxidant systems. We detected higher oxidative stress in urine of diabetic patients. This is also related to progressive glycation of enzymes (Araii et al. 1987) or deficiency of glutathione, ascorbate or alpha-tocopherol.

Kedziora-Kornatowska et al. (1998a) reported that antioxidant enzyme activity was decreased in experimental diabetic rats. The same authors (1998b) reported that SOD and CAT activities were strikingly lower in diabetic patients with uropathy compared to that of diabetic patients without uropathy. Similarly in our study, urine of diabetic patients with urinary tract infection showed intense oxidative stress.

Many inflammatory diseases are characterized by ROS induced tissue damage (Freeman and Crapo 1982). Monocytes and activated phagocytes in diabetics also have an increased capacity to produce superoxide which can inadvertently cause tissue damage (Hiramatsu and Arimori 1988). Okur et al. (2003) reported that urinary MDA levels in the patients characterized by renal infection was significantly higher. Kirschbaum (2001) reported that total antioxidant capacity was lower in urine of patients with renal disease compared to those of healthy persons.

Several studies showed a significant increase in plasma, tissue and urinary MDA in diabetics. (Mc Girr et al. 1985; Godin et al. 1998; Sekeroglu et al. 2000). The mechanisms for increased MDA in diabetes have been investigated and published in several reports (Ramasarma 1982; Young et al. 1992; Sekeroglu et al. 2000). One mechanism for increased MDA level in diabetics is glucose autooxidation. The exact mechanism by which elevated blood glucose leads to lipid peroxidation in

plasma and erythrocytes of diabetic patients is not known (Sekeroglu et al. 2000). The local vascular perfusion disturbances may also be a reason for increased MDA in diabetics.

Our results showed that UTI causes oxidative stress by damaging urinary antioxidant enzymes. Some degree of oxidative stress occurs in most diseases but the question to be answered is whether it makes a significant contribution to the disease pathology (Freeman and Crapo 1982). More than 95% of UTI is caused by a single bacterial species and *E.coli* is the most frequent infecting organism (Gruneberg 1994). Our study is unique in that all isolated organisms were *E.coli*, thereby avoiding the confusion of data due to different microorganisms.

Our results showed that UTI increases severely oxidative stress in DM patients. The risk of UTI is increased in diabetic patients due to acute focal pyelonephritis, necrosis of the papillae and perinephritic abscess formation. Bacteriuria can be seen in 10 - 20% of diabetic patients and *E.coli* is the predominant pathogen in these patients (Powers 2001).

In conclusion, UTI and Type II DM induce oxidative stress through insuffiency of antioxidant capacity and increase lipid peroxidation levels. When these two diseases coincide, they work simultaneously and cause an overwhelming damage. We belive that such patients may be benefited from antioxidant treatments.

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