

# The Effects of Oxidative Stress in Urinary Tract Infection

Ergul Belge Kurutas,<sup>1</sup> Pinar Ciragil,<sup>2</sup> Mustafa Gul,<sup>2</sup> and Metin Kilinc<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Medicine, Kahramanmaraş Sutcu Imam University, 46050 Kahramanmaraş, Turkey

<sup>2</sup>Department of Clinical Microbiology, Faculty of Medicine, Kahramanmaraş Sutcu Imam University, 46050 Kahramanmaraş, Turkey

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We aimed to determine the effects of oxidative stress in urinary tract infection (UTI). One hundred sixty-four urine samples obtained from patients with the prediagnosis of acute UTI admitted to the Faculty of Medicine, Kahramanmaraş Sutcu Imam University, were included in this study. Urine cultures were performed according to standard techniques. Urinary isolates were identified by using API ID 32E. The catalase and superoxide dismutase activity and the lipid peroxidation levels known as oxidative stress markers were measured in all urine samples. Thirty-six pathogen microorganisms were identified in positive urine cultures. These microorganisms were as follows: 23 (63.8%) *E coli*, 5 (13.8%) *P mirabilis*, 4 (11.1%) *K pneumoniae*, 2 (5.5%) *Candida spp*, 1 (2.7%) *S saprophyticus*, and 1 (2.7%) *P aeruginosa*. It was observed that lipid peroxidation levels were increased while catalase and superoxide dismutase activities were decreased in positive urine cultures, compared to negative cultures. We conclude that urinary tract infection causes oxidative stress, increases lipid peroxidation level, and leads to insufficiency of antioxidant enzymes.

## INTRODUCTION

Aerobic organisms possess antioxidant defense systems to deal with reactive oxygen species (ROS), which is a result of aerobic respiration and substrate oxidation. ROS, including hydroxyl radicals, superoxide anions, and hydrogen peroxide, are generated during univalent reduction of oxygen to water. Normally their production is low and the low levels of ROS are necessary for several biological processes, including intracellular differentiation and cell progression, arrest of growth, apoptosis, immunity, and defense against microorganisms. Increased formation of ROS and/or decreased antioxidant defense can be defined as oxidative stress, which may damage biological macromolecules. This possible damage by ROS can be prevented by the endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [1]. Lipid peroxidation is one of the most important expression of oxidative stress induced by ROS. Malondialdehyde (MDA) is an indicator of lipid peroxidation which increases in various diseases. This increase is reflected in enhanced excretion of several MDA derivatives in the urine [2].

Urinary tract infections (UTIs) may appear clinically from asymptomatic bacteriuria to urosepsis. The problem

of UTI spans all age groups beginning with neonates to adults and elderly subjects. Up to date, qualitative tests which include antioxidant enzymes such as CAT and SOD have been used in diagnosis of UTI [3]. To our knowledge, none of the previous researches investigated antioxidant enzyme activities and MDA levels quantitatively both in diagnosis of UTI and as a response to oxidative stress. The present study therefore aimed to determine the effects of oxidative stress in UTI.

## MATERIALS AND METHODS

### *Patients and urine samples*

The study population consisted of patients admitted to the Faculty of Medicine Hospital Kahramanmaraş Sutcu Imam University, between March and November 2004 with symptoms suggesting acute UTI. Inclusion criteria were dysuria, frequency, urgency, and abdominal/flank pain with or without fever. Patients receiving antibiotic therapy were excluded from the study. One hundred and sixty-four urine samples were obtained from patients midstream flow by clean-catch technique and transmitted to Microbiology Laboratory in about half an hour. Urine samples were inoculated into the MacConkey (BD, BBL) and blood agar (BD, BBL, NJ, USA), and incubated at 37°C for 18 hours. A positive result was defined as 10<sup>5</sup> CFU/mL for urine collected from patients. Urinary isolates from positive cultures were identified by using API ID 32E (Biomérieux, France) and conventional biochemical methods.

Correspondence and reprint requests to Ergul Belge Kurutas, Department of Biochemistry, Faculty of Medicine, Kahramanmaraş Sutcu Imam University, 46050 Kahramanmaraş, Turkey; ekurutas@ksu.edu.tr

TABLE 1. Catalase, superoxide dismutase, and malondialdehyde levels in positive and negative urine cultures. † denotes values significantly different from negative urine cultures values;  $P < .01$ . Values were expressed as mean  $\pm$  SDs.

Groups	<i>n</i>	CAT (U/mg creatinine)	SOD (U/mg creatinine)	MDA (nmol/mg creatinine)
Negative urine cultures	128	8.66 $\pm$ 1.97 (3.00–15.09)	17.83 $\pm$ 2.97 (9.98–24.09)	0.84 $\pm$ 0.44 (0.11–1.98)
Positive urine cultures	36	5.15 $\pm$ 1.75 <sup>†</sup> (2.15–10.00)	13.48 $\pm$ 2.33 <sup>†</sup> (9.00–19.88)	3.99 $\pm$ 1.16 <sup>†</sup> (1.99–6.45)

The study was approved by the Medical Ethics Committee of the Faculty of Medicine, Kahramanmaraş Sutcu Imam University, and informed consents were obtained from the patients.

### Biochemical analysis

Spot and 24-hour urine specimens were used for biochemical analysis. To control the urine concentration, data were normalized to urine creatinine concentration. Urinary creatinine was measured in 24-hour urine samples by Dade Behring Dimension RXL autoanalyzer (Germany).

Spot urine samples were diluted with 1:50 physiologic serum (0.9% NaCl) for antioxidant enzyme activities and MDA levels.

### Assay of SOD activity

The method for SOD activity employed xanthine and xanthine oxidase to generate superoxide radicals which react with p-iodonitrotetrazolium violet (INT) to form a red formazan dye which was measured at 505 nm. Assay medium consisted of the 0.01 M phosphate buffer, 3-cyclohexylamino-1-propanesulfonic acid (CAPS), buffer solution (50 mM CAPS, 0.94 mM EDTA, saturated NaOH) with a pH of 10.2, solution of substrate (0.05 mM xanthine, 0.025 mM INT), and 80 U/L xanthine oxidase. SOD activity was expressed as U/mg creatinine.

### Assay of CAT activity

CAT activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm. Assay medium consisted of 1 M Tris-HCl, 5 mM Na<sub>2</sub>EDTA buffer solution (pH 8.0), 1 M phosphate buffer solution (pH 7.0), and 10 mM H<sub>2</sub>O<sub>2</sub>. CAT activity was expressed as U/mg creatinine.

### Assay of MDA

MDA levels were measured by a spectrophotometer. The reaction mixture contained 0.1 mL sample, 0.2 mL of 8.1% sodium dodecyl sulfate, 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 the 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) was added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 minutes, the absorbance of the organic layer was measured at 532 nm. MDA level was expressed as nmol/mg creatinine.

### Statistical analysis

Statistical analysis was carried out with the SPSS-X (release 4.1) program and the differences between patients with and without UTI were analyzed by *t* test.

## RESULTS

A total of 164 patients' urine samples were included in the study: 93 female (56.70%) and 71 male (43.29%), ranging from 23 to 65 years of age (mean  $\pm$  SD, 37.7  $\pm$  13.6 years). Thirty-six pathogen microorganisms were identified in positive urine cultures, which were as follows: 23 (63.8%) *E coli*, 5 (13.8%) *P mirabilis*, 4 (11.1%) *K pneumoniae*, 2 (5.5%) *Candida spp*, 1 (2.7%) *S saprophyticus*, and 1 (2.7%) *P aeruginosa*.

As shown in Table 1, it was observed that MDA levels were increased, while CAT and SOD levels were decreased in positive urine cultures, compared to negative urine cultures ( $P < .01$ ).

## DISCUSSION

Increased formation of ROS and/or decreased antioxidant defense can be defined as oxidative stress, which is widely recognized as an important feature of many diseases. A variety of oxidation products are found in urine and thought to mirror local and systemic oxidative stress [4]. Acute terms of various diseases accompany many inflammatory conditions and influence the endogenous antioxidant enzyme activities. In the present study we thought that UTI may cause an oxidative stress, and also the antioxidant enzymes measured quantitatively were depleted in response to oxidative stress. Kirschbaum reported that total antioxidant enzyme activity was lower in patients with acute renal disease compared to those of control urine specimens [4]. In our study, although there was not renal damage, CAT and SOD activities were decreased in the urine samples of patients with UTI compared to negative urine cultures. These results showed that UTI may cause oxidative stress by consuming urinary antioxidant enzymes and it is possible to say that urinary

antioxidant enzymes are not enough to prevent the oxidative stress in UTI. According to our study, overproduction of free radicals generated during infection may lead to the low levels of antioxidant enzymes. Since the start of antibiotic therapy according to the culture results may take time, antioxidant agents such as vitamin C may be given promptly in addition to the antibiotic therapy. In a study that investigated the effect of vitamin C on UTI, Foxman and Chi found that vitamin C, an antioxidant, gives protection against UTI [5].

MDA, an indicator of lipid peroxidation in cell and tissues, has been reported to be mutagenic in bacterial and mammalian cells. In the recent years, MDA was used as a marker of oxidative stress [2]. Urinary MDA is found in increased quantities in some diseases such as thalassemia, renal failure, and pancreatic disease [6]. In this study we found that urinary MDA level was 4.75 times higher in positive urine cultures compared to negative urine cultures and may indicate the existence of oxidative stress. Since MDA test can be obtained before the results of cultures taken in urinary tract infection, it may be used as an ancillary diagnostic tool and may contribute to the initiation of treatment without waiting for the culture results.

As a conclusion, UTI may cause oxidative stress and increase lipid peroxidation level leading to insufficiency of antioxidant enzymes. We believe that patients with UTI may benefit from antioxidant treatments in addition to antibacterial treatment.

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