

Biochemical Analysis of Malondialdehyde and Antioxidant Enzymes in Patients with Urinary Tract Infections

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ABSTRACT

Objective: This study aimed to evaluate the impact of gram-negative uropathogenic bacteria on oxidative stress and the antioxidant defense system in adult patients with urinary tract infections (UTIs). **Methods:** We analyzed 165 blood and urine samples from UTI-diagnosed patients admitted to Tuz General Hospital in Salahaddin, Iraq. Urine cultures were performed following standard microbiological procedures, using API ID 32E for the identification of urinary isolates. Serum was obtained by centrifuging blood samples and was then assayed for oxidative stress markers, including malondialdehyde (MDA) levels, and for antioxidant enzyme activities such as glutathione reductase and superoxide dismutase. **Results:** Out of 110 gram-negative bacterial isolates identified, the prevalence was as follows: *Escherichia coli* (58; 52.72%), *Klebsiella pneumoniae* (30; 27.27%), *Pseudomonas aeruginosa* (12; 10.81%), and *Proteus mirabilis* (10; 9.09%). Patients with positive urine cultures showed significantly elevated serum MDA levels and reduced activities of glutathione reductase and superoxide dismutase compared to those with negative cultures, indicating oxidative stress induced by UTI. **Conclusion:** UTIs caused by gram-negative bacteria are associated with significant oxidative stress, manifested by increased MDA levels and decreased antioxidant enzyme activity in the serum of affected patients. These biochemical changes underscore the pathological impact of UTIs and highlight the need for strategies that mitigate oxidative stress in this patient population. This study emphasizes the importance of oxidative stress markers as potential diagnostic and therapeutic targets in the clinical management of UTIs.

Keywords: Antioxidants, Glutathione Reductase, Malodialdehyde, UTI, *E.coli*

INTRODUCTION

Antioxidants are chemical compounds that donate protons or electrons to free radicals, making them more stable [1]. The production of free radicals is linked to damage in membranes, enzymes, and the cellular nuclear material [2]. The ability of antioxidants The immune system and other bodily systems rely on reducing these extremely reactive free radicals to preserve the structural integrity of their cells and tissues [3]. In the absence of an adequate amount of antioxidants to counteract reactive oxygen species (ROS), various detrimental processes such as biomolecular oxidation, including Some of the things that can happen include lipid peroxidation, protein impairment (such as carbonylation of amino acid residues), oxidative damage to specific DNA and RNA nucleotides, enzyme inhibition, and the start of cell death [4]. There are two types of antioxidants: exogenous and endogenous antioxidants. Exogenous antioxidants can be derived from natural sources such as vitamins, flavonoids, and certain mineral compounds [5]. Antioxidant defence works by detecting and neutralising free radicals, preventing their formation, and transforming harmful free radicals into less harmful ones. Interrupting the chain reaction of secondary oxidants, Preventing the synthesis of inflammatory mediators and secondary hazardous metabolites, Molecular damage repair and activation and enhancement of the body's natural antioxidant defences [6]. The existing evidence strongly indicates that the majority of urinary tract infections (UTIs) stem from a single bacterial origin primarily present in the gut, with infrequent occurrences produced by several kinds of microbes, like fungus or viruses. Among bacterial agents, gram-negative bacteria emerge as the predominant contributors to UTIs, encompassing species such as *Escherichia coli*, *Klebsiella spp.*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* [7]. In order to combat the reactive oxygen species (ROS) that are byproducts of aerobic respiration and substrate oxidation, aerobic organisms have evolved defence mechanisms. Hydroxyl radicals, superoxide anions, and

hydrogen peroxide are components of reactive oxygen species (ROS) that are produced when oxygen is reduced to water. Low levels of reactive oxygen species (ROS) generation are normal and necessary for many biological processes, including cellular differentiation, growth arrest, apoptosis, immunity, and defence against microbes. Damage to biological macromolecules can occur as a result of oxidative stress, which occurs when antioxidant defences are reduced or ROS generation is excessive. Luckily, our bodies have antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) that help protect cells from ROS-induced damage. Elevated excretion of several MDA derivatives in urine is a reflection of the increased amount of malondialdehyde (MDA), a biomarker of lipid peroxidation, a major symptom of oxidative stress caused by reactive oxygen species (ROS) [8].

MATERIALS AND METHODS

Patients of the Study

This research was conducted at Tuz General Hospital, Salahaddin, Iraq. It took place from November 2022 to March 2023. Each patient's history, including their name, age, gender, recurrent infection, and whether they were currently undergoing treatment or not (with no antibiotic intake for 3 days before collecting the specimen for culture), was documented using a questionnaire form.

Urine Sample Collection

In this investigation, a total of 165 urine samples were gathered from men and women from 20 to 60 years old, rural and urban who had urinary tract infections (UTIs) as diagnosed by the doctor Turan. 165 male and female patients with UTIs were randomly selected to participate in the study. After voiding into sterile disposable containers, clean-catch midstream fresh urine samples weighing 30 ml were

collected. The MacConkey and Nutrient agar from Liofilchem, Italy, were cultured with urine samples, and then incubated at 37°C for 18 hours.

When testing patient urine, a sensitivity level of 105 CFU/mL was considered positive. The identification of urinary isolates from cultures that tested positive was done using API 20E (Biomérieux, France) in conjunction with more traditional biochemical techniques.

Blood Sample Collection

The blood samples were collected and placed in tubes containing a gel separator. Following a clotting period of 2 hours at room temperature, the tubes were centrifuged at a speed of $1000 \times g$ for 20 minutes. The freshly prepared serum samples for the assay were stored in separate portions at a temperature of -80°C in order to assess glutathione reductase, superoxide dismutase activity, and malondialdehyde levels in the future.

Glutathione Reductase Assay

Sandwich enzyme immunoassay is the testing principle utilized in this kit. An antibody specific to Glutathione Reductase (GR) has been pre-coated onto the microtiter plate that is included in this kit. Before

using a biotin-conjugated antibody specific to Glutathione Reductase (GR), standards or samples are added to the correct microtiter plate wells. After that, each well of the microplate is incubated after adding Avidin conjugated to Horseradish Peroxidase (HRP).

Not all wells will change colour after adding TMB substrate solution; only those with Glutathione Reductase (GR), biotin-conjugated antibody, and enzyme-conjugated Avidin will. Spectrophotometric measurement of the colour change at a wavelength of $450 \text{ nm} \pm 10 \text{ nm}$ is used to end the enzyme-substrate reaction. After that, we compare the optical density (OD) of the samples with the standard curve to find the concentration of Glutathione Reductase (GR) in the samples.

Calculation of GR Result

In order to illustrate the accepted understanding of the Human GR Standard and the associated optical density (O.D.) measurements, logarithmic scales on both the x- and y-axes are required. By plotting the sample's O.D. on the Y-axis, we may determine the concentration of human GR in the sample. Multiplying this reading by the dilution factor yields the starting concentration (Figure 1).

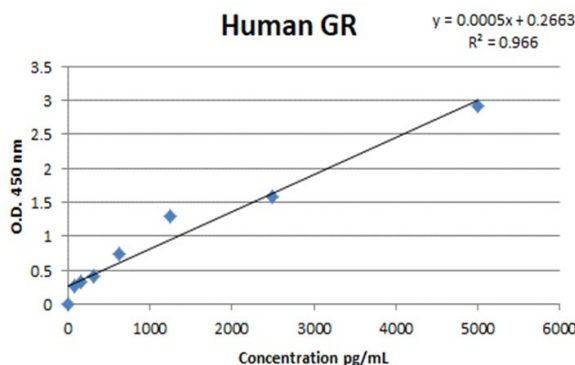


FIGURE 1: Standard Curve for Glutathione Reductase (GR)

Human Superoxide Dismutase Assay

Sandwich enzyme immunoassay is the testing principle utilized in this kit. An antibody specific to Superoxide Dismutase (SOD) has been pre-coated onto the microtiter plate in this kit. A biotin-conjugated antibody specific to Superoxide Dismutase (SOD) is used in conjunction with the correct microtiter plate wells to detect standards or samples. The next step is to incubate each microplate well with Avidin-conjugated Horseradish Peroxidase (HRP). Wells containing Superoxide Dismutase (SOD), biotin-conjugated antibody, and enzyme-conjugated Avidin will be the only ones to show a color change with the addition of TMB substrate solution. Spectrophotometric

measurement of the color change at a wavelength of $450 \text{ nm} \pm 10 \text{ nm}$ is used to end the enzyme-substrate reaction. After that, we compare the optical density (OD) of the samples with the standard curve to find the concentration of Superoxide Dismutase (SOD) in the samples.

Calculation of SOD Result

Representing established understanding of Human SOD Standard and its associated optical density (O.D.) readings involves using logarithmic scales on both the x-axis and the y-axis. The quantity of human SOD present in the specimen is ascertained by plotting the sample's O.D. on the Y-axis. The initial concentration can be derived by multiplying this value with the dilution factor (Figure 2).

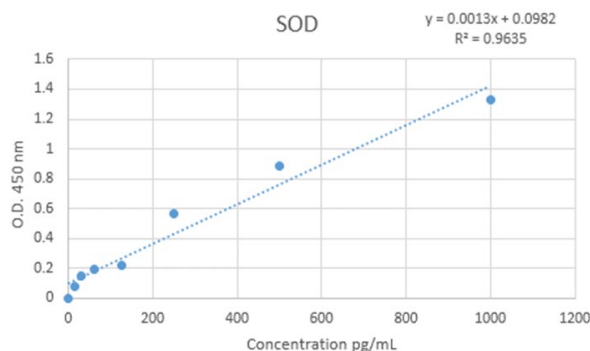


FIGURE 2: Standard Curve for Human Superoxide Dismutase (SOD)

Malondialdehyde Assay

Competitive inhibition enzyme immunoassay is the method used in this test. This kit includes a microtiter plate that has already been coated with Malondialdehyde (MDA) protein. A biotin-conjugated antibody specific to Malondialdehyde (MDA) is used in conjunction with the correct microtiter plate wells after standards or samples have been introduced to them. The next step is to incubate each microplate well with Avidin-conjugated Horseradish Peroxidase (HRP).

Upon the addition of the TMB substrate solution..... The injection of sulphuric acid solution stops the enzyme-substrate reaction, and the spectrophotometric measurement of the colour change is taken at a wavelength of $450\text{nm} \pm 10\text{nm}$. Next, we compare the optical density (OD) of the samples with the standard curve to find the concentration of malondialdehyde (MDA) in the samples.

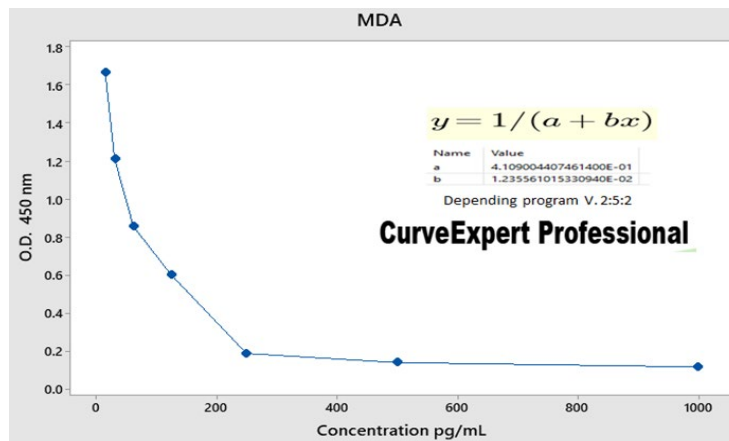


FIGURE 3 : Standard Curve for Human Malondialdehyde (MDA)

Furthermore, the Pearson correlation test was utilized to examine the associations between each parameter among patients with urinary tract infections (UTIs) and those without UTIs.

Results & Discussion

With ages ranging from 20 to 60, the study comprised urine samples from 165 patients, 93 of whom were female (56.36%) and 72 of whom were male (43.64%). Urine cultures that came out positive

Calculation of MDA Result

Representing the acknowledged understanding of the Human MDA Standard and its associated optical density (O.D.) readings requires employing logarithmic scales for both the horizontal (x-axis) and vertical (y-axis) axes. The quantification of human MDA present in the specimen is determined by plotting the sample's O.D. on the vertical axis. The initial concentration is derived by multiplying this value by the dilution factor (Figure 3)

Statistical Analysis

Statistical analysis was conducted using the SPSS software. The t-test was employed to assess the distinction in serum glutathione reductase (GR), superoxide dismutase (SOD) activities, and malondialdehyde (MDA) levels between healthy individuals and patients with urinary tract infections (UTIs).

contained 110 different types of gram-negative bacteria. These microorganisms were as follows: 58 (52.72%) *Escherichia coli*, 30 (27.02 %) *Klebsiella pneumoniae*, 12 (10.81%) *Pseudomonas aeruginosa*, and 10 (9.45%) *Proteus mirabilis*.

As indicated in (Table 1), it was noted that levels of MDA demonstrated an increase, whereas levels of GR and SOD exhibited a decrease in the serum of individuals with positive urine cultures, when compared to healthy individuals (p-value < 0.05) .

Table 1: Serum Levels of Glutathione Reductase, Superoxide Dismutase, and Malondialdehyde in Individuals with Positive Urine Cultures and Healthy Individuals (p-Value > 0.05). Values were Expressed as Mean $\pm$ SDs

Group	N	Glutathione Reductase pg/ml	Superoxide Dismutase pg/ml	Malondialdehyde pg/ml
- Healthy People	20	4333 $\pm$ 1559	79.1 $\pm$ 61.0	397 $\pm$ 294
-Patient With Positive Urine Culture for Gram -Ve Bacteria	110	3271 $\pm$ 1291	34.2 $\pm$ 12.0	914 $\pm$ 393
-P.Value		p<0.05	p<0.05	p<0.01

Oxidative and nitrosative stress occur when there is an elevated production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), or when the mechanisms responsible for antioxidant activity decrease. This leads to an imbalance between oxidants and antioxidants [9]. Numerous investigations have indicated that persistent inflammation constitutes the primary factor in the development of various diseases. Within the processes of inflammation, unchecked reactive elements damage lipids within cellular membranes, impacting both their permeability and the configuration and performance of proteins. This process results in DNA damage and mutations, which significantly influence the oxidative

condition of cells and reduce the efficacy of antioxidant defense mechanisms [10]. In the current research, we hypothesized that urinary tract infections (UTI) could induce oxidative stress. Furthermore, the antioxidant enzymes that were quantitatively assessed demonstrated depletion in response to this oxidative stress. Kurutas EB et al. [8] observed that levels of lipid peroxidation were elevated, whereas the activities of catalase and superoxide dismutase were diminished in urine cultures that tested positive compared to those that tested negative [11]. In Delshad M et al.'s [12] systematic review, a total of 50 articles from PubMed and 6 records from Google Scholar were initially identified. However, only 8 articles met the

inclusion criteria for further assessment. Three additional papers were excluded due to either unavailability of full texts or language irrelevance. Ultimately, five articles that fully satisfied the inclusion criteria were selected for data extraction. The results of these studies revealed a significant decrease in antioxidant levels among patients with urinary tract infections (UTIs), while markers of oxidative stress showed an increase. Soleimani G's observed a reduction in the total antioxidant enzyme capacity of serum in children with urinary tract infections (UTI) when compared to control serum samples. In contrast to serum samples from healthy people with negative urine cultures, we observed reduced activity of glutathione reductase (GR) and superoxide dismutase (SOD) in UTI patients' samples during our experiment. According to these results, a UTI may cause oxidative stress by lowering the levels of antioxidant enzymes. It is conceivable to draw the conclusion that the antioxidant enzymes already in existence may not be adequate to combat the oxidative stress linked to UTIs. Based on our findings, the reduced levels of antioxidant enzymes may be caused by the overproduction of free radicals during infection. Antioxidants such as vitamin C may be given in addition to antibiotic treatment, as starting antibiotic medication based on culture findings may require some time. Okoroiwu G [13] found that vitamin C offers

some protection against UTI in a study examining the effect of vitamin C on UTI. Malondialdehyde (MDA), a widely recognized end product arising from lipid peroxidation, experiences up-regulation in reaction to an elevated presence of free radicals. Therefore, the amount of MDA acts as a prominent indicator of oxidative stress. Elevated levels of plasma MDA are observed in certain conditions, including thalassemia, renal failure, and pancreatic disease [14, 15].

In our investigation, we discovered that the serum MDA level was 2.3 times greater in patients with positive urine cultures compared to the serum of healthy individuals with negative urine cultures [16]. This elevated level of MDA could potentially signify the presence of oxidative stress. As conclusion, urinary tract infections (UTIs) can induce oxidative stress and elevate Malondialdehyde levels, resulting in a deficiency of antioxidant enzymes. We propose that patients suffering from UTIs could potentially gain advantages from antioxidant therapies alongside antibacterial treatments. Moreover, the MDA test shows promise as an additional diagnostic tool for UTIs, as it can be conducted prior to receiving culture results, thus potentially facilitating the commencement of treatment without waiting for culture results.

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