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Effects of Oxidative Stress in Urinary Tract Infection

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Abstract

Background: Urinary tract infections (UTIs) may appear clinically from asymptomatic bacteriuria to urosepsis. UTI is known to involve oxidative stress. However, the reports on role of antioxidant in patients with UTI are scanty. Hence the present study was undertaken to assess the activity of free radicals and antioxidant defense\mechanism in patients with variety of UTI.

Method: A total 30 patients with UTI were selected based on clinical examination and urine analysis. Age, sex matched 44 healthy normal controls were also included in the study. Plasma lipid peroxidation (LPO) and activities of key erythrocyte enzymes such as superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) and plasma levels iron enzymatic antioxidants such as ascorbic acid, transferrin, ceruloplasmin, uric acid, bilirubin and albumin were measured in UTI patients and compared with normal controls.

Results: The plasma LPO were significantly higher in patients with UTI (16.21±2.66 nmol/ml) than in normal control (9.53±1.93 nmol/ml), ($P<0.001$). The erythrocyte enzymatic defense (SOD, CAT and GPx) significantly lowered in UTI patients when compared to normal controls ($P<0.001$). Transferrin level was significantly increased in patients with UTI (234.71±36.76 mg/dL) than normal control (204.81±38.89 mg/dL), ($P<0.001$). Whereas the level of plasma ascorbic acid, albumin, ceruloplasmin were significantly decreased in patients with UTI when compared to normal controls ($P<0.001$). Plasma bilirubin and uric acid levels increased in UTI patients than normal control, but which was not statistically significant, indicating bilirubin and uric acid are not changed UTI patients.

Conclusion: UTI may cause oxidative stress and increase lipid peroxidation level leading to insufficiency of antioxidant enzymes.

Keywords: Urinary tract infections; Oxidative stress; Free radicals; Lipid peroxidation; Glutathione peroxidase; Superoxide dismutase; Ceruloplasmin

Introduction

Urinary tract infections (UTI's) are among the most common condition encountered in office practice, hospitals, and extended care facilities. About 50-60% of adult women reports that they had UTI at some time [1]. UTI's are important complications in pregnancy, diabetes, polycystic renal disease, renal transplantation and structural and neurological conditions that interfere with urine flow. The infectious process may involve the kidney, renal, pelvis, ureters, bladder and urethra as well as the adjacent structure such the perinephric fascia prostate and epididymis [2].

Most common microorganisms in UTI are: -Gram-negative bacteria (Escherichia coli, Klebsiella Pneumoniae, Enterobacter aerogenes, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobactor species, Serratia marcescens, Providencia stuartii and rettgeri) and Gram-positive bacteria (Staphylococcus bacteria, staphylococcus aureus, coagulase-negative, ataphylococci group B and D streptococci, yeasts, candida albicans). Urinalysis usually shows pyuria, bacteriuria and gross or microscopic hematuria. Chronic pyelonephritis implies prolonged or recurrent infection, with progressive destruction of renal parenchyma [3].

During the process of phagocytosis, the respiratory burst occurs releasing superoxide radical into the phagosome to kill the bacteria [4]. It is also released into the tubular lumen. Superoxide dismutase is present in essentially all tissues within body, but its concentration in urine is very low. However, the action of this toxic form of oxygen is unopposed and effect of its metabolite's hydrogen peroxide, hydroxyl radical, myeloperoxide or singlet oxygen, all these reactive oxygen species (ROS) can be toxic to the surrounding cell membranes. Release of phagocyte lysozymes into the tubular lumen might also be expected to be toxic to surrounding, renal tubular cells. Tissue death is rapid and a single inoculation of bacteria into the kidney of either the infant or adult primate leads to loss from 20 to 30% of the function of involved kidney in the absence of therapy. Although lipid peroxidation is one of the most important expressions of oxidative stress induced by ROS [5]. Up to date, qualitative tests which include antioxidant enzymes such as CAT and SOD have been used in diagnosis of UTI [6]. In the present study, plasma LPO and antioxidant defense was studied in patients with UTI.

Materials and methods

A total 30 indoor patients of age ranged from 20-58 years with UTI were included in the study group. The patients were selected based on clinical examination, laboratory urinalysis test and culture test. According to Pezzol test for UTI, this test is based on the number of WBC/µl in urine which detects 95% of UTI and it has very good correlations with WBC excretion rate the gold standard for assessing pyuria [7]. Age and sex matched 44 normal healthy controls including students and staff from Krishna Institute of Medical Sciences Karad, who free from diseases including, diabetes mellitus, infection, hypertension, coronary artery disease, atherosclerosis and any renal dysfunction and no history of smoking were also included.

Blood samples were collected from above selected patients and normal controls. Blood was collected by

vein puncture in heparin bulb with the help of plastic disposable syringe. They were centrifuged soon, and the plasma was separated. The separated plasma was taken into another container. Plasma was used for estimation of lipid peroxide (LPO), ascorbic acid, bilirubin, uric acid, ceruloplasmin, transferrin, and albumin. The erythrocytes were harvested and were washed thrice with isotonic saline and supernatant was discarded. This process removed more than 99% of the white cells. Packed cell volume (PCV) was used to prepare hemcolysate. To the packed cells 1.5 volume of distilled water was added. The erythrocytes lysed by mixing on vortex mixer for 5 minutes. Mixing gives clear hemolysate. Hemolysate was collected and used to measure hemoglobin concentration on sysmex 20. The hemoglobin concentrations were adjusted to 10 gm% by appropriate dilution of the hemolysate with distilled water. This concentrated hemolysate (10 gm%) was used to measure SOD activity. Rest of hemolysate diluted to 5 gm% with distilled water and used for determination of catalase and glutathione peroxidase activity.

Lipid peroxidation was measured by colorimetric method [8]. The measurement of erythrocyte enzymes such as superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) was done [9]. Also, the estimation of plasma ascorbic acid was done [10]. The most common method for the clinical determination of bilirubin is the coupling of serum bilirubin with diazotized sulfanilic acid. (*p* diazobenzene sulfonic acid) to produce azobilirubin dye. Total bilirubin concentration was determined using Malloy and Evelyn method [11]. Ultrasensitive detection of uric acid in serum of patients [12]. Determination of plasma albumin was done [13]. Ceruloplasmin concentration was estimated [14]. Transferrin was assayed and indirectly using, plasma total iron level. Plasma iron was measured by dye binding colorimetric method [15] using ferrozine.

Statistical analysis

Statistical analysis was carried out with the SPSS version 20. The differences between patients with UTI and normal controls were analyzed by t test. P value less than 0.001 was considered statistically highly significant and P value <0.05 was significant.

Observations and Results

In the present study, 30 patients with urinary tract infection were included based on clinical examination and urine analysis. Out of these UTI patients 11 were males and 19 were females.

The plasma lipid peroxides (LPO) measured as thiobarbituric acid reactive substances (TBARS) were significantly higher in patients with UTI than in normal control subjects (P<0.001). The erythrocyte enzymatic defense (SOD, CAT and GPx) significantly lowered in UTI patients when compared to normal controls (P<0.001). Transferrin level was significantly increased in patients with UTI (234.71±36.76 mg/dL) than normal control (204.81±38.89 mg/dL), (P<0.001). Whereas the level of plasma ascorbic acid, albumin, ceruloplasmin were significantly decreased in patients with UTI when compared to normal controls (P<0.001). Plasma bilirubin and uric acid levels increased in UTI patients than normal control, but which was not statistically significant, as shown in table 1.

Table 1: The values of plasma LPO, SOD, CAT, GPx, TFR, CER, Albumin, Ascorbic acid, bilirubin and Uric acid in patients with UTI

Parameters	Normal controls (n=44)		Patients with UTI (n=30)	
	Mean ± SD	Range	Mean ± SD	Range
LPO nmols / ml	9.53 ±1.93	6.86 -14.06	16.21 ± 2.66*	10.94-20.22
SOD unit/mg Hb	22.53 ± 3.06	16.66- 26.78	10.58 ± 2.36*	7.15-16.50
CAT#	543.82 ± 69.67	465.76 - 823.92	195.87 ± 44.63*	135.40-301.15
GPx Unit/L	576.04 ± 100.65	460.24-685.80	257.75 ± 42.69*	172.88-336.18
TFR mg/dL	204.81 ± 38.69	142.00 - 321.18	2.34.71 ± 36.76*	180.00- 288.12
CER mg/dL	38.47 ± 6.86	26.65-52.90	28.73 ± 6.30*	18.19-37.18
Albumin gm/dL	3.94 ± 0.49	3.00- 4.50	3.34 ± 0.35**	2.80 -3.90
Ascorbic acid mg/dL	0.57 ± 0.17	0.28-0.96	0.22 ± 0.083*	0.09-0.43
Bilirubin mg/dL	1.08 ± 0.25	0.80-1.80	1.17 ± 0.40**	0.9 - 2.00
Uric Acid mg/dL	3.16 ± 0.64	1.60-4.62	3.29 ± 0.92*	2.00-7.20

The activity of catalase expressed as mm of H_2O_2 decomposed /mgHb/min; * NS = Not Significant

Plasma LPO levels were elevated in UTI compared to the controls. The erythrocyte SOD, GPx and CAT activity most important index of enzymatic antioxidant defense was decreased in UTI disorders compared to controls. The plasma level of ceruloplasmin was lowered and transferrin level was elevated in UTI patients compared to controls. However, the mean level of plasma albumin, ascorbic acid, bilirubin was decreased while uric acid level was increased in UTI patients compared to controls as depicted in figure 1.

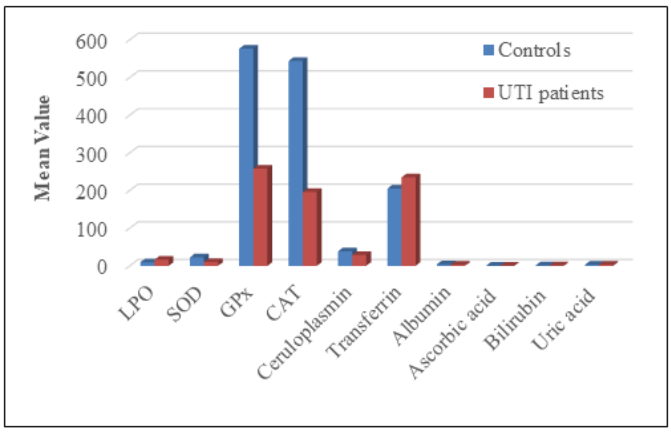

Figure 1: Comparison of LPO, erythrocyte enzymatic defense (SOD, CAT and GPx) and iron enzymatic antioxidants between cases and controls

Discussion

The renal infection group including the diseases such as acute/chronic pyelonephritis, focal bacterial nephritis, renal/perinephric abscess, pyonephrosis and renal tuberculosis represents a wide spectrum of interrelated conditions. Like renal ischemia and platelet aggregations, smooth muscle constrictor, activation of prostaglandin synthetase, and many toxic effects are mediated factors produced the macrophages [16]. In present study, activity of free radicals and antioxidant defense\ mechanism was studied in the 30 patients with variety of UTI. The results obtained from these patients showed higher levels of plasma LPO, indicating, increased oxidative stress and decline antioxidant defense potential as indicated by low levels of erythrocyte enzymes SOD, GPx and CAT, and plasma concentration of ascorbic acid, albumin and ceruloplasmin. The antioxidants like transferrin,

bilirubin and uric acid were significantly increased in patients with UTI. The plasma proteins such as transferrin (TFR), ceruloplasmin (CER) and albumin are the important chelator protein of transition metals which prevents participation of transition metals in the chain reaction of free radicals.

The mechanism underlying the increased oxidative stress in the UTI remains unclear. In current study, the significant increase in plasma LPO levels suggests a free radical mediated oxidative stress in UTI. The major cause of oxidative stress is the body's immune system. Although toxic, traumatic injury may also lead to the release of lysosomes and oxydiazing enzymes. Both neutrophils and activated macrophages release hydrogen peroxide and other compounds [17]. The pathology is related to oxidation of nucleic acids, chromosome breaks and peroxidation of unsaturated fatty acids in the cell membranes. Limited chromosomal damage can be repaired whereas extensive DNA damage promotes apoptosis of the affected cell. On the other hand, extensive membrane damage may lead to cell necrosis with the release of lysosomal enzymes, which further generates OFR [18].

The markedly decreased antioxidant status may be due to increased free radical activity as well as some other factors in UTI. The excessive production of OFR occurs by polymorphonuclear leukocyte activation during infections or by a prooxidant effect of tumor necrosis factor a produced by activated macrophage [19]. Early studies have shown that neutrophils, sensitized monocytes macrophages and eosinophils may be activated by both particulate and non-particulate stimuli to generate oxygen free radicals. These cells are a major source of O_2, H_2O_2, OH and hypochlorite (HOCl) [20]. Microbial infection activates the respiratory burst in phagocytes. During this respiratory burst oxygen consumption is increased. It is associated with activation of membrane NADPH oxidase, which catalyses the reduction of molecular oxygen to superoxide radical and oxygen free radicals by electron transfer from NADPH [21].

$2O_2 + NADPH \rightarrow 2O_2 + NADP^+ + H^+$

As NADPH oxidase is located on the surface of cell. O_2 generation without vacuole formation results in the generation of O_2 into the extra cellular medium. The clinical manifestations of this are yet poorly characterized deficiency, are recurrent infections, low grade fever and raised acute phase proteins [22]. Respiratory burnt is also activated by the synthesis of prostaglandin, thromboxane and arachidonic acid, metabolite of cytochrome P450 monooxygenase, throughout the renal vascular and tubule. The metabolites of P450 system include epoxides of archidonic acid such as monohydroxy - eicosatetraenoic acid (HETES) and hydroxy peroxy - eicosatetraenoic acid (HETES) and omega oxidation product pathways generates free radicals [23]. These are possible sources of free radicals, generation in the UTI.

In the UTI the markers of free radical activity such as lipid peroxides (malondialdehyde) was estimated. It was found that the level of MDA was significantly increased when compared to normal healthy controls, indicating increased free radical activity. And this increased activity could be any of the above processes, which becomes dominant or goes out of control. The possible reason in increasing free radical activity may be due to decreased antioxidant defense capacity in UTI.

The erythrocyte enzyme such as SOD, GPx and CAT plays a major role in the metabolism of OFR. The activities of these enzymes were significantly lowered in

the patients with UTI. The reason for weakening of erythrocyte enzymatic defense is not clear. Data from several epidemiological studies suggests that dietary antioxidant vitamins and trace minerals have protective effect against infection especially in advanced stages of the disease [24]. The beneficial effect of selenium supplementation on superoxide dismutase activity compared with the base line values unaffected, however glutathione peroxidase activity and glutathione status increased. The decreased antioxidant enzyme activity could be due to increased utilization of antioxidant micronutrients because of increased oxidative stress rather than to inadequate dietary intake [25]. However, OFR associated changes in immunoglobin during infection and inflammatory condition gives rise to formation of immune, complexes with IgA and IgM. These complexes can, in turn, activate cells to produce OFR and proteolytic enzymes which, both independent and synergistically, may degrade the SOD, GPx and CAT in UTI [26]. And subsequently decrease in the activities of SOD, CAT and GPx in erythrocytes. In present study, there was significant decrease in activity of SOD, CAT and GPx when compared to normal healthy controls, indicates-that decreased antioxidant defense capacity in-patients with UTI might have resulted due to any of the above mechanism.

The transition metal binding proteins such as transferrin, ceruplasmin and albumin plays an important role in preventing redox activity of transition metal, by chelating free metal ions, that escape during cell death or i:h rapid turnover. Transferrin level was increased in UTI patients than the normal healthy control. In this study, the transferrin was derived as calculated transferrin from the total iron level in plasma. In infection and inflammatory conditions, neutrophil derived oxidants such as superoxide radical release iron from ferritin into extra cellular fluid. Therefore, local increase in free iron may contribute to local oxidative stress. Ceruloplasmin increases iron binding to transferrin (Fe+++) by ferrooxidase activity and prevents Fe+2 from reacting with hydrogen peroxide. In present study, ceruloplasmin levels were found to be significantly lowered in-patients with UTI. Early studies suggests that decreased ferroxidase activity of ceruloplasmin in oxidative stress result from free radical damage [27] The present results also favor this hypothesis. Albumin a weak copper binding protein also showed significant decrease inpatient with UTI. The albumin clearance may be increased resulting albuminuria during infection and inflammatory condition of renal system. This could be possible cause for decrease in albumin level in UTI. The antioxidant vitamin ascorbic acid was. significantly decreased in patients with UTI than that of the normal control subjects. The possible cause of reduction in plasma ascorbic acid was increased consumption of the ascorbic acid in chronic oxidative stress as occurs in UTI [28]. Secondly in the inflammatory conditions low levels of ascorbic acid may result due to oxidative convention of ascorbic acid into dehydro ascorbic acid (DHA). This DHA fails to regenerate to ascorbic acid in chronic oxidative stress [21]. The plasma, bilirubin levels of UTI patients were found to be unchanged. There Was no difference between normal healthy control group and patients with UTI. The uric acid levels were significantly higher than normal control in the UTI patients. In the oxidative stress reperfusion injury may induces the xanthine oxidase system to produce. Uric acid causes slightly higher levels of uric acid in the patients with UTI.

The findings of the present study revealed that the free radicals are the common causative denominator of UTI disorder. The clinical relevance of the results suggests that the antioxidants can be used in the therapeutic intervention for better progress of the renal diseases. Although the finding of this study may be compelling clinicians for prescribing antioxidant vitamin or other micronutrients, their use as therapeutic agent is limited only as an adjunct to the mainline therapy. In general, the golden rate is prevention is better than cure and thus health care professionals should encourage a diet rich in fresh fruit, vegetables and whole grain cereals to the general population.

Conclusion

The results of present study, suggest that decreased antioxidant defense is responsible for increased free radicals and free radical activity in UTI, which increases the oxidative stress and is associated with pathological changes in renal system. Increased oxidative stress may be the major cause for decreasing activity of antioxidant enzymes. The exact mechanism and the role of decreased antioxidant defense is poorly understood. We hypothesized that increased oxidative stress may damage lysosomal membrane. Consequently, release of cathepsin into cytoplasm might occur. The increased activity could hydrolyzing enzyme, proteins and increased free radical activity damages the genes of antioxidant enzyme. The low activity of antioxidant enzymes could be due to deficiency of trace elements required for their activity or could be due to deficiency of or co-substrate required for their action. We believe that patients with UTI may benefit from antioxidant treatments in addition to antibacterial treatment.

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