OXIDATIVE STRESS IN CKD PATIENTS WITH URINARY TRACT INFECTIONS

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INTRODUCTION

Reactive oxidative species (ROS) play crucial roles in gene activation, cellular growth, and modulation of chemical reactions in the cell and function as major components of the defense against bacteria and viruses provided by neutrophils (phagocytes) and as agents responsible for dilation of blood vessels (eg, NO.).

Urinary tract infections (UTIs) are among the most prevailing infectious diseases. Renal sources for ROS are activated macrophages, vascular cells, and various glomerular cells. ROS may affect cells of the host organism, especially at sites of inflammation, in addition to playing a role in the defense system against other agents. This effect plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, which can contribute to proteinuria and other conditions.

Reduced GSH is the most important intracellular scavengers of free radicals. GSH serves as a reductant in oxidation reactions resulting in the formation of GSSG. Thereby decreased GSH levels and increased GSSG levels may reflect depletion of the antioxidant reserve. GSH/GSSG ratio is the most useful indicator of oxidative stress in cells and tissues. 8-OH-dG (8-hydroxy-2-deoxy Guanosine) is produced by the oxidative damage of DNA by reactive oxygen and nitrogen species. Although more than 20 base lesions have been identified, only a fraction of these have received appreciable study, most notably 8-oxo-2'deoxyguanosine. F2-isoprostane, which is a nonenzymatic, free radical-catalysed isomer of cyclooxygenase-derived enzymatic products of arachidonic acid, can be found in both urine and plasma. Isoprostane increased level was associated with hepatorenal syndrome, rheumatoid arthritis, atherogenesis and carcinogenesis. Several of these compounds possess potent biological activity, as evidenced mainly through their renal vasoconstrictive effects, and have short half-lives. Both human and experimental studies have indicated associations of isoprostanes and severe inflammatory conditions, ischemia-reperfusion, CKD, diabetes and atherosclerosis.

AIM OF STUDY: to determine markers of oxidative stress in patients with moderte CKD and urinary tract infection.

MATERIAL AND METHODS

The analyses were conducted in three groups of individuals. Group of healthy controls recruited from among the pacients with biochemical parameters in normal range and without any history of renal disease (age: 56.56 ± 4.95 years). The individuals in the control group were not on any prescribed medications (including anti-oxidants). Group CRF with infections produced by E. coli and with infections with other ethiology. Patients with uremia pre-dialysis (n = 104) were recruited from the Constanta County Hospital, (age: 65.57 ± 9.36 years). All participants gave fully informed consent to participation in the study.

Samples for microbiological tests were collected and processed using standard microbiological protocols. The isolated strains were identified on device Vitek2 on GN identification cards and the sensitivity test was perfomed on AST GN 27 cards. We assessed reduced and oxidized glutathione (GSH) content in red blood cells, and determine the GSH/GSSG ratio. The quantitative determination of the total amount of glutathione (GSH+GSSG) employs the enzimatic method first reported by Tietze. Briefly, the reaction of GSH with Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid: DTNB) give rise to a product that can be quantified spectrophotometrically at 412 nm. The reaction is used to measure the reduction of GSSG to GSH. The rate of reaction is proportional to the GSH and GSSG concentration. The GSH/GSSG ratio was calculated by dividing the difference between GSH and GSSG concentration by the concentration of GSSG: $\text{Ratio} = (GSH - 2GSSG) / GSSG$

For determining levels of 15-isoprostane F2 (the best characterized isoprostane) in urine samples we used a competitive enzyme-linked immunoassay (ELISA). Briefly, urine spot-samples are mixed with an enhanced dilution buffer that essentially eliminates interference due to non-specific binding. The 15-isoprostane F2 in the samples or standards competes with 15-isoprostane F2 conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody specific for 15-isoprostane F2 coated on the microplate. The HRP activity results in color development when substrate is added, with the intensity of the color proportional to the amount of 15-isoprostane F2-HRP bound and inversely proportional to the amount of unconjugated 15-isoprostane F2 in the samples or standards.

Quantitative and sensitive detection of 8OHdGuanosine have been carried out using ELISA. ELISA conventional method is excellent in light of its sensitivity and great convenience. It is a competitive assay that can be used for the quantification of isoprostane in urine. The plates are coated with specific polyclonal antibodies. The tracer consist in a enzyme linked with the isoprostane. The analytes in the samples are competes with tracer for binding to antibodies coated on the microplate. The activity of the enzyme results in color development when substrate is added, with intensity of the color inversely proportional to the amount of unconjugated analyte in the samples.

RESULTS

The urinary tract infections diagnosed during hospitalization included Gram-negative rods (*E. coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aerogenes, Enterobacter spp., Alcaligenes faecalis, Acinetobacter baumannii*) and Gram-positive cocci (*Enterococcus faecalis, Enterococcus faecium*). Because *E. coli* represents 75% from bacteria identified we consider the group of patients with *E. coli* infections. The patients with other microorganisms: *Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Alcaligenes faecalis Enterococcus faecalis, Enterococcus faecium* are part of the third group.

A significant difference between patients with renal infections and healthy controls was found in the levels of GSH/GSSG ratio both for *E. coli* and other ethiological agents. An decrese in GSH/GSSG ratio ($p<0.001$) was taken as indicative of oxidative damage. The GSH/GSSG ratio is considered the best determines the antioxidant capacity of cells, and any decrease suggests a strong oxidant effect (fig. 1).

Compared with controls, the patients with other renal infections had a significantly higher level of urinary 8-OH-dG ($p<0.002$).

A significant positive correlation was found between GSH/GSSG ratio and 8OHdG for patients with different type of urinary infections ($r= 0.96$) (fig. 2). In addition, positive correlation were found between isoprostane and endothelin level in patients with *E. coli* infections ($r = 0.91$).(fig. 3) These may be attributable to the inflammatory process, but vascular endothelial dysfunction is a very important event in CRF, and oxidative stress induced by vasospasm is the most characteristic sign that reflects this dysfunction. Ischaemia and reperfusion injury during renal disease can generate reactive oxygen species that may result in vascular endothelial damage. In addition, isoprostane itself is a potent vasoconstrictor, has platelet pro-aggregant functions and stimulates endothelial cells to bind monocytes, which may promote vascular obliteration, inflammation and spasm. Therefore, the finding that high isoprostane levels correlated with endotheline level that excessive oxidative stress is related to vascular damage in CRF.

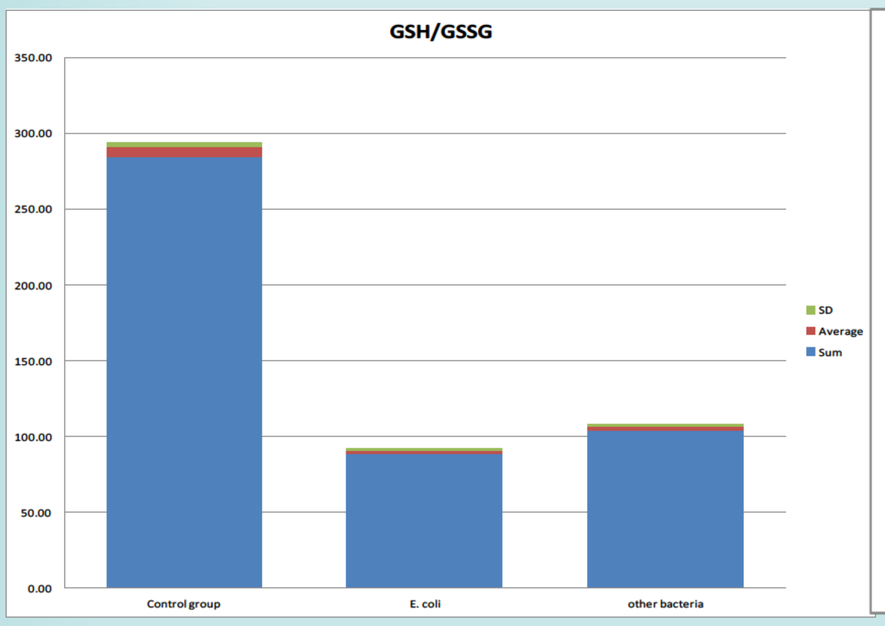

Figure 1. Average level, standard deviation, for GSH/GSSG ratio for healthy control and patients with infections

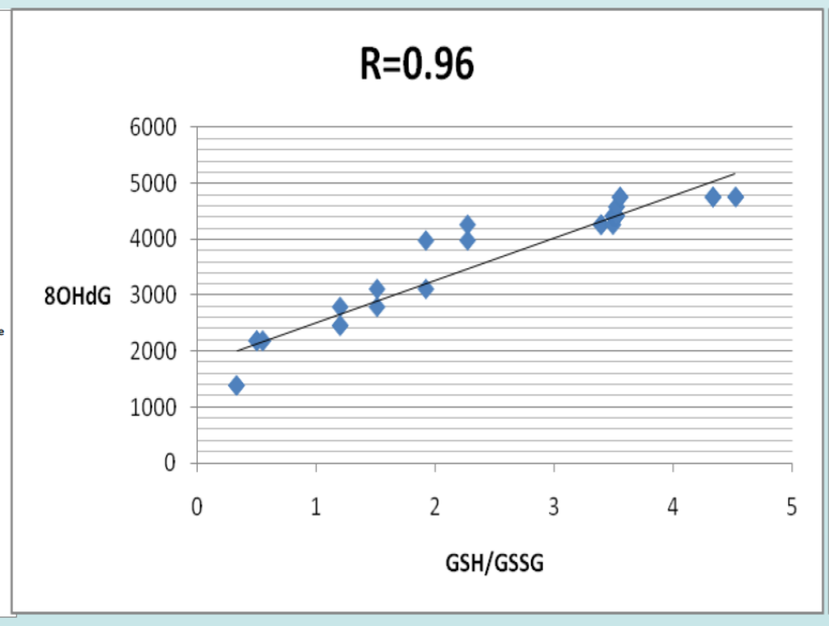

Figure 2. Correlation between GSH/GSSG ratio and 8OHdGuanosine

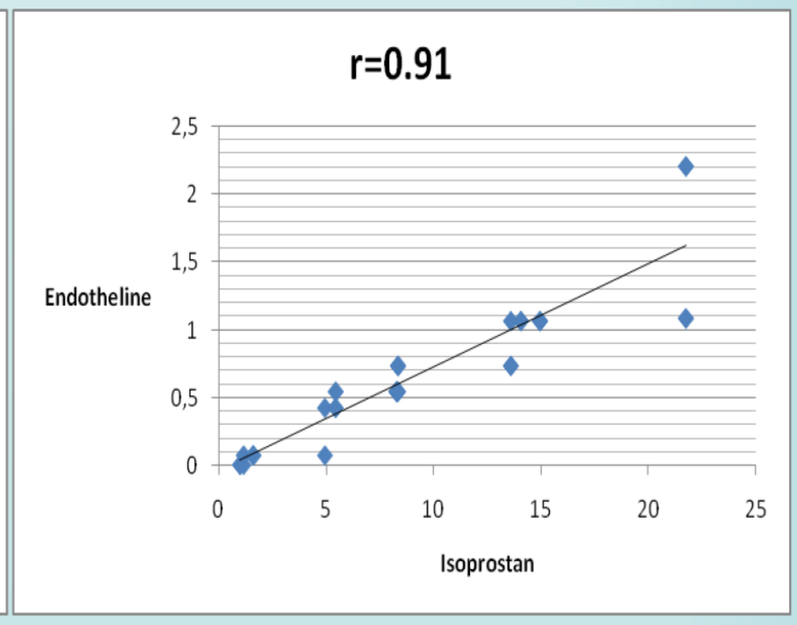

Figure 3. Positive correlation were found between isoprostane and endothelin level in patients with E. coli infections

CONCLUSION:

Markers of endothelial oxidative stress (high isoprostane levels) are more increased in CKD patients with urinary tract infections with E coli, in comparison with CKD patients associating UTI's caused by other germs, showing a different pathway of action and aggravation of oxidative stress in these special population. Starting from this observation, we would search for a special therapeutical approach in E. Coli infections, that maybe will minimize inflammation in this group of patients, and delay the progression to end-stage renal disease.

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