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# URINARY SUBCLASSES OF GLUTATIONE-S-TRANSFERASE ( and ) AS AN INDICATOR OF EARLY DAMAGES IN HUMAN KIDNEY TUBULAR CELLS

**Boncheva M.[1], G. Nikolov[2], T. Gruev[2]**

*[1]Medical University of Varna, Chair of Clinical Laboratory, Bulgaria*
*[2]University Clinic of Clinical Biochemistry, Skopje, Macedonia*

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## ABSTRACT

**The aim of this work was to obtained information about the possibility of differentiating (with - and -GST) between cellular damage at different levels of the tubular system in specific forms of acute renal failure (ARF) - ischemic vs. obstruction. The study focused specifically on differencing between acute allograft rejection and cyclosporineA induced nephrotoxicity. Patients and methods. Twenty two patients, hospitalized at the Department of Nephrology, Clinical centre-Skopje, suffering from ARF with the average age 48.1±5.6 years were studied. Forty five recipients with renal allograft were followed daily from the time of transplantation until discharge from hospital and then twice weekly for a total 3 months. Urine sample were obtained from patients with ARF as an ischemic tubular necrosis and acute obstructive renal insufficiency and patients with renal transplantation. The concentration of - and -GST in urine were measured following the method of quantitative EIA (Biotrin Int.LTD). The serum samples from transplanted patient were measured for creatinine and CystatinC. RESULTS. Significant differences of p-GST values between ischemic ARF and acute renal obstruction were observed, while they were insignificant for the values of -GST. There were no significant differences for -GST values of all three samples in both groups with ARF. In the cases of acute rejection after transplantation, the release of -GST into the urine is limited, while that of the -GST isoenzyme is extensive. In contrast to the case of acute rejection, in the early stages of CsA induced nephrotoxicity a significant amount of -GSTs excreted into the urine, but no significant elevation of urinary -GST is seen.**

**Key words:** ARF, -GST, -GST, acute rejection, CsA nephrotoxicity

## INTRODUCTION

Alpha and pi glutathione-s-transferase ( -GST and -GST) are proteins (ligands) normally found in high concentrations in the cells of the proximal and distal renal tubules respectively (4) and has been shown that they are released into the urine following damage such as acute tubular necrosis (ATN) (15) nephrotoxicity (16) and rejection (1). The urinary levels of each GSTs subclass (isoenzymes) are specifically related to the site of renal injury. The GSTs are present in the cytoplasm in soluble form and they are distinct from the structural, immunological and catalytically point of view. The -GST has a molecular weight of 51 kD, the -GST- 47kD, while the major form"μ"-form, weighs 53 kD. Nephrotoxicity, usually affecting the proximal tubule, normally results in increased levels of -GST (unique cytosolic protein encountered in the proximal epithelia, readily released from damaged cells) into the urine. Toxic event or rejections which affect mainly the distal tubule, the thin loop of Henley and the collecting ducts, are characterized by urinary release of pi GST( unique ligand present into the distal tubular and collecting cells, highly specific and sensitive as a marker of distal tubular damage). Ischemic lesion (reperfusion injury), witch affects the whole tubular system, leads to release of both isoenzymes (б-and -GSTs). Furthermore, urinary excretion of GSTs indicates renal damage before elevated serum creatinine and Cystatin C levels have been detected. The distal tubular function may be difficult to investigate, without strongly related specific and sensitive marker like -GST. Classically, acute kidney rejection were found to induce the release of -GST into the urine. By combining the results for and -GST with other markers of renal damage (neutral endopeptidase, metalloproteinases, aminopeptidase A and M, в2 microglobulin, retinol binding protein), new attractive areas of basic and clinical re-

**Address for correspondence:**
*G. Nikolov*
*University Clinic of Clinical Biochemistry, Skopje, Macedonia*
*Tel: ++ 389 78 443 792*
*e-mail:* n.goran@ymail.com

search may be opened, that would enable to demonstrate more precisely the site of renal injury along the tubular segments. In earlier investigation it was proposed that the urinary level of -GST loud be used for diagnostic purposes, since in connection with cyclosporine A induced nephrotoxicity this protein is released from its specific location in the cells of the proximal tubules into the urine(4,15,16). Glomerular and tubular cells damage may be provoked also by immunotoxins (immune complexes, proinflammatory cytokines), reactive oxygen metabolites, tubular hyperfunction in addition to renal vasoconstriction and arterial hypertension.

**The aim** of this work was to obtain information about the possibility of differentiating (with -and -GST) between cellular damage at different levels of the tubular system in specific forms of acute renal failure (ischemic vs. obstruction), as well as to clarify the association between the clinically well defined situations (global ischemic ATN, obstructive acute renal insufficiency) of kidney damage, with the presence of different GSTs into the urine. Also in the present investigation we have examined the urine from transplanted patients with tubular damage and evaluated the release of GST- in the relationship to the clinical evaluation. This study focused specifically on differentiating between acute transplant rejection and cyclosporineA (CsA) –induced nephrotoxicity. In addition, we wanted to obtain information about the possibility of differentiating between cellular damage at different levels of the tubular system as well as to investigate possibilities of differentiating between glomerular and tubular diseases.

## PATIENTS AND METHODS

### *Patients*

Twenty two patients, hospitalized at the Department of Nephrology, Clinical centre, Skopje suffering from ARF (12 females and 10 males) with the media age 48.1±5.6 years were studied. Forty five recipients of renal allograft (31 female and 14 males) were followed daily from the time of transplantation until discharge from hospital and there after twice weekly for a total 3 months. All patients ( two from them with initial post-transplant tubulopathy) were subjected to evaluation using clinical observation, laboratory tests and morphological examination (mainly urinary tract ultrasonography). The Ethics Committee approved the study protocol and every participant gave a fully informed approval to take part in the study. The urine (night) samples were collected during a period of 8 hours at room temperature and their volume measured. Samples kept in the refrigerator at $4^{o}$ for 5 days showed no significant decrease in enzymes activity, but the room temperature gradually decreased the enzyme contents (restriction to 35% of the original amount) in the same period of time. The GSTs stability was not appreciably affected by pH (pH range 5.3-8.5). Control urine was obtained from 30 students undergoing check up and found to be healthy. Samples from each patient with established diagnosis were chosen for analysis and presentation the third and ninth day of polyuric phases of ARF, and two months after admission to the hospital. Recipients of renal allograft with a starting serum creatinine level above 700 mmol/L, initial oligouria and obvious hyperhydratation (cases of initial ischemic tubulopathy wit ATN) were treated like other patients with ARF requiring dialysis. CsA was not included in the therapy as long as ARF was not completely resolved.

### *Methods*

Urines were collected from control participants, patients wit ARF and patients after transplantation in different etiology. Sample were centrifugated (2000g for 15 min). GST's concentrations were measured (expressed in g/L) with a quantitative enzyme immunoassay designed for urine analysis (Biotrin NEPHKIT- and -Biotrin International, Dublin, Ireland) in microtitre wells coated with anti-pi; anti GST IgG, on the ELISA reader at 450 nm (reference:620 nm), and $25^{0}$C by monitoring the increase of absorbance because of the release of chromogenic substrates. The resultant color intensivity is proportional to the amount of isoenzymes present in the samples. The reference range (control patients) was: 3.7±2.3(1.4-6.0) g/L for - and 13.7±5.3 (8.1-18.7) g/L for -GST respectively. The concentration of creatinine in serum and urine were determined with kinetically Jaffe methods (9). CystatinC concentration was determined immunoturbidimetic with the DAKO test (reference range 1.2 ± 0.4 mg/L).

### *Statistics*

All data were expressed as average ± SD of the number of experiments. The statistical significance was evaluated by Student's t-test using one-way ANOVA together with Dunca's range test. In all instances, the criterion for statistical significance was established at 0.05 before testing.

### *Results*

The results are presented in tables as average value and standard error. Demographic characteristics of investigated patients are presented in the (Table 1).

No sex related differences in urinary / GSTs excretion were detected in both group of patients with acute renal failure. The group with acute obstructive post renal insufficiency was significantly older than the group with ischemic ATN 58.6±13.1 vs. 43.3±14.5 ( $p<0.05$). Significant differences of -GST values between ischemic ARF and acute renal obstruction were observed, while they were insignificant for the values of - GST. Further on, the GST values in ischemic injury group were significantly lower two months after admission than in the group with acute renal obstruction. The higher values of standard errors for mean values of -GST, need further investigations to elaborate these various results (Table 2).

The laboratory results obtained with the patents with stable renal function, acute rejection and CsA nephrotoxicity is presented in Table 3. The serum and urine samples ana-

*Table 1. Demographic characteristics of investigated patients with ARF and post transplanted complications.*

| Diagnosis | Average age (X±SD) (range in years) | Sex F | Sex M | Number of cases |
|---|---|---|---|---|
| Ischemic acute tubular necrosis (ATN) | 43.3±14.5 | 9 | 6 | 15 |
| Acute obstructive post renal insufficiency | 58.6±13.1 | 3 | 4 | 7 |
| Acute rejection after transplantation | 38.2±8.5 | 13 | 5 | 18 |
| CsA nephrotoxicity | 41.5±13.5 | 4 | 2 | 6 |

*Table 2. - and - Gluthatione transferase in ARF of different etiology.*

| | Ischemic renal injury(ATN) | | Acute post renal obstruction ARF | |
|---|---|---|---|---|
| | -GSTμ/L | -GST μg/L | -GST μg/L | -GST μg/L |
| 3-d day | 137.3±30.6* | 24.0±5.4* | 470.2±107.7** | 25.8±5.9* |
| 9-th day | 95.4±21.6* | 16.2±3.7* | 245.1±52.7** | 12.6±2.8* |
| 2 months after admission | 15.1±1.2 | 4.6±1.7 | 123.0±28.6 | 8.0±1.8 |

*p< 0.001; **p<0.0001

lyzed were collected on the same day for each patient. In comparison with the stable patients (Table 4) , which had a serum creatinine level 148.0± 45.0 μmol/L and Cystatin C 1.65± 0.38mg/L, those having acute rejection had a serum creatinine level 250.6±86.5μmo/L (p<0.001) and Cystatin C 3.86±0.95 mg/L ( p<0.001). In patients diagnosed as having CsA-induced nephrotoxicity had a serum creatinine 221.7±60.2 mol/L, also significantly higher that the stable group (p<0.001).The urinary level of GST- changed characteristically in connection with the conditions examined. In contrast of GST- , the - form was detectable in control patients 13.4±5.3 μg/L and did not differ significantly from the level found in the stable group (15.8±6.1 μg/L). In acute rejection we was found 348.2±165.6 (p<0.001), but no observed changes in patient with CsA nephrotoxicity. The excretion of GST- was quite different from that of GST- . The concentration in control group was 3.7±2.3 g/L, in the stable 4.1±1.35 g/L, but significantly increased in patients with CsA-induced toxicity (26.2±2.9, p< 0.001).

*Table 3. - and -Gluthatione transferase in patients with renal transplantation.*

| -GST g/L | | -GST g/L |
|---|---|---|
| Control values | 3.7±2.3 | 13.4±5.3 |
| Stable Graft function | 4.1±1.3 | 15.8±4.2 |
| Acute rejection | 5.3±1.95 | 348.2±176.5** |
| CsA induced toxicity | 26.2±2.9* | 16.2±2.9 |

* p< 0.001; **p< 0.0001

*Table 4. The concentration of serum creatinine and CystatinC in patients with ARF and renal*

| Creatinine ( mol/L) | | Cystatin C (mg/L) |
|---|---|---|
| ARF 3-d day | 421.8±98.0** | 3.45±1.2** |
| ARF 9-th day | 314.5±75.1** | 2.38±0.95** |
| ARF 2 months after admission | 163.4±68.0** | 1.46±0.55* |
| Stable graft function | 148.0±45.0* | 1.65±0.38* |
| Acute rejection | 250.6±86.5** | 3.86±0.95** |
| CsA-induced toxicity | 221.7±60.2** | 2.65±0.76** |

*p < 0.05; ** p< 0.001

## DISCUSSION

In earlier studies, RIA and EIA usefulness was demonstrated for analyzing the level of -GST protein in urine for diagnosis of certain kidney conditions, mainly associated with ischemic or toxic proximal tubular cells destruction (such as dishaemodinamic, ATN and CsA induced nephrotoxicity). Thus, the detection and quantification of both urinary GSTs can be used to monitor a large portion of the tubular system. In the process damages the entire tubular system, both GSTs would be excreted into the urine, as in the case of acute tubular necroses where the serum creatinine level increased in both type of ARF, but better correlation has been found with -GST. In obstructive ARF enzymuria is ordinarily present from the previously

obstructed kidney. In this study we have analyzed two groups of patients with ARF (with ischemic ATN and obstructive acute renal insufficiency). As it was expected, global ATN affected both tubular structures (proximal and distal) with significant -/ -GST enzymuria. However, the acute renal obstruction provoked slight б-GST urinary excretion with extensive and persistent -GST enzymuria. The persistency of elevated -GST into the urine in patients with obstructive ARF, may suggest perhaps a more serious initial lesion and/or persistency of other factors, such as partial obstruction with or without parenchymal infection and medullar necrosis, some of witch have not been recognized and considered. The final statement and explanation does not oppose the accepted approach that "both increase and decrease in the urinary levels of GSTs occur rapidly". Furthermore, the increased enzymuria often precedes (few days) the rise of other functional parameters, for e.g. the level of serum creatinine and Cystatin C. On the other hand hand, the absence of GSTs into the urine in an obstructive process may signify previous chronic fibrosclerotic kidney lesions of the distal tubules and collecting ducts, before the attack of obstructive ARF, with the previously diminished tubular cells reserves of GSTs (especially -GST) (1). In the other hand the results obtained in investigated transplanted patients demonstrate the possibility of differential diagnosis. From the practical point of view, the differencing diagnosis between the acute kidney transplant rejection (limited release of alfa GST into the urine, while that of the -GSTis extensive) and CsA associated nephrotoxicity in kidney transplant patients, become easier (a significant б-GST enzimuria vs. no elevation in urinary -GST). These findings are in agreement with present investigation, that the initial phase of such toxicity only the proximal tubules are injured; the distal tubule being affected only at a later stage. However, a distinction between CsA induced renal toxicity and graft rejection is not possible to be made only on the basis of serum creatinine and Cystatin C levels, since this parameters increases to an equal extent in both conditions. Many efforts were also made to predict the condition of transplanted graft during the very early postoperative period. The parameters that may quantify the damage before and after transplantation are currently regaining importance. Determination of enzymes, nucleotides, electrolyte release and functional and morphological status did not prove useful to predict the outcome of the transplantation (14,3,12,5,13,18,17,7). During ischemic state, the plasma membrane integrity in damaged, and its permeably is also altered (8,2). In case of acute reaction in our investigation the release of -GST into the urine is limited (5.3±1.95μg/L) while that of -GST isoenzymes is extensive (348.2±176.5μg/L; $p<0.001$) . The early stages of CsA nephrotoxicity a significant amount of -GST is excreted into the urine (26.2±2.9 g/L; $p<0.001$), but no significant elevation of -GST is seen (16.2±2.9μg/L). Our result showed that a distinction between acute rejection and CsA nephrotoxicity is not possible to make on the basis of serum creatinine and Cystatin C levels, since this parameters increases to an equal extent in both conditions. After all, the changes in the concentration of Cystatin C is much more rapid and earlier suggest initial renal dysfunction (6,9,10). This findings is in agreement with investigation of Sibley RK. at el (14), Bergstrand A. at el (3) and Mihatsch MJ. at el (12) that in the initial phase of such toxicity, only the proximal tubules damaged and the distal portion being affected only at a later stage. Assay of the urinary levels of these two glutathione transferases differentiates between CsA induced toxicity and acute rejection, a distinction witch is not possible to make on the basis of serum creatine and Cystatin C levels, since this parameters increases to an equal extent in both conditions.

## CONCLUSION

In conclusion Acute Renal Failure is associated with elevated urine levels of GSTs. While the ischemic ARF is characterized with predominantly -GST urinary excretion (proximal tubular lesion), obstructive kidney insufficiency presents mainly -GST urinary elimination (distal and collecting tubules' injury). By assaying urinary levels of both - and -GST in transplanted patients, one can differentiate between damage in various portions of the renal tubular system.

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