α and π- glutathione transferases as a markers of tubular cells dysfunction in acute renal failure patients

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Abstract

Twenty-two patients with acute renal failure(ARF), 15 cases with renal ischemic injury and 7 patients with acute obstructive nephropathy, were analysed in the period of hospitalisation (3, 9 day of polyuric phase) and two months after admission of the patients to the hospital. The urinary levels of the both enzymes: α and π-glutathione transferases (α and π-GST) were detected in the urine samples following the method of quantitative EIA (Biotrin Int. LTD) in μg/l. The results are presented in the following table:

	ischemic renal injury		acute renal obstruct.	
	π-GST	α-GST	π-GST	α-GST
3 th day	135.3± 143.5	24.0± 25.5	470.0± 505.0	25.8± 27.7
9 th day	095.4± 101.1	16.2± 17.2	245.1± 247.4	12.6± 13.2
2 mont. after admiss.	005.1± 005.4	07.6± 08.1	123.0± 134.1	08.0± 08.3

As a conclusion, the urinary α-GST and π-GST are a sensitive markers for clinical evaluation of renal tubular injury. After the two months, the enzymes activity is stabilised in both groups of patients. The persistence of elevated π-GST levels in the urine in a few patients with obstructive ARF, may suggest a more serious lesions or evolution to chronic nephropathy.

Introduction

Acute kidney injury (AKI) is currently recognized as the preferred nomenclature for the clinical disorder formally called acute renal failure (ARF). This transition in terminology served to emphasize that the spectrum of disease is much broader than that subset of patients who experience failure requiring dialysis support (1,2)This new nomenclature underscores the fact that kidney injury exists along a continuum: The more severe the injury, the more likely the overall outcome will be unfavorable. The Acute Kidney Injury Network (AKIN), which was formed recently in an effort to facilitate improved care of patients who are at risk for AKI, described AKI as "functional or structural abnormalities or markers of kidney damage including abnormalities in blood, urine, or tissue tests or imaging studies present for less than three months." AKI is associated with the retention of creatinine, urea, and other metabolic waste products that are normally excreted by the kidney. Although severe AKI may result in oliguria or even anuria, urine volume may be normal or even increased (3,4)Recent epidemiologic data suggest that the progress observed in the understanding of the pathophysiology of AKI and in the clinical care of patients with AKI has failed to yield

commensurate impro-vements in clinical outcomes. AKI has been reported to complicate 1% to 7% (5,6) of all hospital admissions and 1% to 25% of intensive care unit (ICU) admissions (7).Over the past 50 years, mortality rates of patients with AKI in the ICU have remained high, at approximately 50% to 70%. Although there is some indication that mortality rates may be falling, the incidence of AKI has increased greatly over time (8). A recent large international study of the epidemiology and outcome of AKI in critically ill adult patients reported an overall in-hospital mortality rate of 60% (9). Of those who survived to hospital discharge, 13% remained dialysis-dependent. In a smaller retrospective study of 267 adult AKI survivors requiring acute renal replacement therapy, renal insufficiency persisted in 41% and overall 5-year survival postdischarge was 50% (10-15) Accessible markers of AKI can be components of serum or urine or can be imaging studies or any other quantifiable parameter. The urine has yielded the most promising markers for the early detection of AKI and further charac-terization is anticipated, which will qualify these markers as useful tools for the earlier diagnosis, identification of mechanism of injury, and assessment of site and severity of injury. Hopefully, one or more of these biomarkers, either alone or in combination, will prove to be useful in facilitating early diagnosis, guiding targeted intervention and monitoring disease progression and resolution.Alpha and pi glutathione s-transferases (αGST AND πGST) are proteins (ligands) normally found in high concen-trations in the cells of the proximal and distal renal tubules respectively (16) and has been shown that they are released into the urine following damage such as acute tubular necrosis (ATN) , nephrotoxicity (17,18) and rejection(19). The urinary levels of each *GSTs* subclass (isoenzyme) are specifically related to the site of renal injury. The third major classe of *GTSs (s.c. "*μ" *GST*) is not present in relatively high amount into the human renal tissue. The *GSTs* are present in the cytoplasm in soluble form and then they are distinct from the structural, immune-logical and catalytical point of view. The enzymes are present as dimers of two subunits of equal size. The α*GST* has a molecular weight of 51 kD, the π*GST*-47 kD, while the major "μ"-form, weighs 53 kD. The μ*GST* is present in only 50% of the population. Nephrotoxicity, usually affecting the proximal tubule*,* normally results in increased levels of α*GST* (unique cytosolic protein encountered in the proximal epithelia, readily released from damaged cells) into the urine. Toxic events or rejections which affect mainly the distal tubule, the thin loop of Henle and the collecting ducts, are characterised by the urinary release of π*GST* (unique ligand present into the distal tubular and collecting cells, highly specific and sensitive as a marker of distal tubular damage). Ischaemic lesion (reperfusion injury), which affects the whole tubular system, leads to release of both isoenzymes (α/π GSTs). Furthermore, urinary excretion of *GSTs* indicate renal damage before elevated serum creatinine levels have been detected (20) From the clinical point of view, urinary α*GST* is a very useful test for proximal tubular cells damage caused by many agents like: x-ray contrast media, aminoglycosides such as gentamycin, amikacyn, netilmycin, heavy metals (Pb,Hg, Cd), different enviromental chemicals and drugs (anti cancer medicaments, polymixine, some diuretics, cephalosporines and espe-cially: Cyclosporin A). Glomerular and tubular cells damage may be provoked also by immunotoxins (immune complexes, pre/infla-mematory cytokines), reac-tive oxigen meta-bolites, tubular hyperfu-nction, low grade oxidized lipids (LDL-oxidation) in addition to renal vasoconstriction and arterial hyper-tension*.* As a consequence, sublethal lesion produces "apototic" release of membrane proteins which are shed into the urine at an increased rate, as a tool for early detection of renal cell damage.The distal tubular function may be difficult to investigate, without strongly related specific and sensitive marker like π*GST.* Classically, acute kidney rejection, foscarnet and lithium intoxication*,* was found to induce the release of π*GST* into the urine. By combining the results for π and α *GSTs* with other markers of renal damage (neutral endopeptidase and metalloproteases, amino-peptidases A and M, dipeptidyl pepti-dases, chip 28 water channel, β2 microglo-bulin, retinol binding protein), new attractive areas of basic and clinical research may be opened, as it may now be possible to demon-strate more precisely the site of renal injury along the tubular segments. Micro/al-buminuria without *GSTs* enzymuria*,* correlates mainly with primary glomerular disease. Histoche-mical detection of isoenzy-mes, measu-rement of the release of α and π *GST* from slices and cell cultures, has been shown to be a perfect means of invest-tigating the metabolism of potentially nephrotoxic substances, drug candidates and environ-mental (pollution) or Industrial agents. Immunohistochemical staining of a kidney biopsy specimen for *GSTs,* utilises formalin fixed slices incubated with the antiserum, and the antigen-antibody complex visualises by the peroxidase-antiperoxidase technique using quantitative image analysis(21). The aim of this work was to obtain information about the possibility of differentiating between cellular

damage at different levels of the tubular system in specific forms of acute renal failure (ischaemia vs obstruction), as well as to clarify the association between the clinicaly good defined situations (global ischaemic ATN, obstructive acute renal insufficiency) of kidney damage, with the presence of different *GSTs* into the urine.

Matherial and Methods

Assay principle

"Biotrin's" (Biotrin ltd, Dublin, Ireland) urinary α GST EIA (Cat.No bio 66NEPHA) and πGST EIA (Cat.No bio 69NEPHPi) are a quantitative solid phase enzyme immune-oassay.The procedure is based on the sequential addition of sample, enzyme conjugate and substrate to microliter wells coated with anti α/π GST Ig G.The resultant colour intensity is proportional to the amount of isoenzymes present in the samples. Total assay time is 2 or 2.5 hours respectively. The GSTs, isolated in a higly purified form, have been used for the production of polyclonal antisera and subsequently EIA sensitive techniques, for quantification of this ligands.Measuring range: the practical measures utilise a previously created calibration curve with a basic range between 0-40 (3-100)μg/l respectively for α and π GSTs in urine diluted 1/2. This range may be extended by increasing sample dilution.Then, the routine measurements could be performed regularly in the range 0.5 to 1000 (or more) μg/l.The reference ranges are as concentration (mean$\pm$SD) 3.5$\pm$11.1μg per liter for αGST, and-13.7$\pm$42.6 μg per liter for πGST respectively.Our own reference range (control group,No 30) was:3.7 $\pm$ 2.3 (1.4-6.0) and 13.4$\pm$ 5.3(8.1 - 18.7)μg/l, for α and π GST respectively.

The urine samples were obtained from patients with acute renal failure (ARF) as an ischaemic acute tubular necrosis and acute obstructive renal insufficiency at the Department of Nephrology, Clinical Centre, Skopje.

Twenty two (22) patients, not pretreated with nephrotoxic drugs, suffering from ARF (12 females and 10 males) with a median age of 48.1 $\pm$ 5.6 years (range: 19 - 76), were studied.

The patients were examined upon admission to the hospital, to confirm the diagnoses of deteriorating renal function. All patients (two from them with initial posttransplant tubulopathy) were subjected to evaluation using clinical observations, laboratory tests and morphological examinations (mainly urinary tract ultrasonography).The urine (night) samples were collected during a period of 8 hours at room temperature (from 10 h p.m. to 6 h a.m.) and their volumes measured. Thereafter the samples were delivered to the Department of Clinical Chemistry as soon as possible, in spite of known *GSTs* proteins stability and immunological reactivity even at room temperature for more than 24 h[5]. Samples kept in the refrigerator at 4°c for 5 days showed no significant decrease in enzymes activity, but the room temperature gradually decreases the enzyme contents (restriction to 35% of the original amount) in the same period of time (120 hours). The *GTSs* stability is not appreciably affected by pH (range: 5.3 - 8.5). Control urine was obtained from students undergoing a chek up and found to be healthy.

Patients

From each patient where diagnosis was established, a samples were chosen for analysis and presentation the third and nineth day of polyuric phase of ARF, and two months after admission of the patients to the hospital (No 20). Recipients of renal allograft (No 2) with a starting serum creatinine level above 700 μmol/l, initial oliguria and obvious hyperhydratation (cases of initial ischaemic tubulopathy with ATN) were treated like an other patients with ARF requiring
dialysis). The *Cs A* was not included in the therapy so long as the ARF was not completelly resolved.The patients have demonstrated a normal global renal function at the end of two months after admission to the hospital. But, following ATN, soluble membrane antigens may cross the contraluminal basement membrane barrier and diffuse into the renal interstitium, inducing a chronic, progressive and aseptic interstitial nephritis.

Results

The results are presented in tables as mean value and standard error.

Table 1. Demographic characteristics of investigated patients

Diagnosis	Mean age (X±SE), (range in years)	Sex F* M*	Number of cases
Ischaemic acute tubular necrosis (ATN)	43.3 ±14.5	9 6	15
Acute obstructive postrenal insufficiency	58.6±13.1	3 4	7
Total	48.1±15.6 (19-71 years - F) (39-76 years - M)	12 10	22

No sex related differences in urinary α/π GSTs excretion was detected in both groups of patients. The group with acute obstructive postrenal insufficiency was significantly older than the group with ischaemic ATN 58.6±13.1 vs 43.3 ±14.5, $p<0.05$.

Table 2. α/π Glutathione transferases in ARF of different etiology

	Ischaemic renal injury		Acute renal obstruction	
	π-GST	α-GST	π-GST	α-GST
3-d day	135.3±30.6	24.0±5.4	470±107.7	25.8±5.9
9-th day	095.4±21.6	16.2±3.7	245.1±52.7	12.6±2.8
2 months after admission	005.1±1.2	07.6±1.7	123.0±28.6	08.0±1.8

Significant differences of π-GST values between ischaemic ARF and acute renal obstruction are observed, while it was not significant for the values of α-GST. Further on, the π-GST values in ischaemic injury group was significantly lower at two months after admission then in the group with acute renal obstruction. There was no significant differences for α-GST values in both groups at all three samples.

The higher values of standard errors for the mean values of π-GST , need further investigations to elaborate so different results.

Discussion

Earlier, it was demonstrated the usefulness of RIA and EIA analysis of the level of the α*GST* protein in urine for diagnosis of certain kidney conditions, mainly associated with ischaemic or toxic proximal tubular cells destruction, like dishaemodynamic ATN and C*s A* induced nephrotoxicity. Thus, the detection and quantification of both urinary *GSTs* can be used to monitor a larger portion of the tubular system. From the practical point of view, the differential diagnosis between the acute kidney transplant rejection (limited release of α*GST* into the urine,

while that of the π*GST* is extensive) and *Cs A* associated nephrotoxicity in kidney tranplant patients, become more easier (a significant α*GST* enzymuria vs no elevation in urinary π*GST*). This findings are in agreement with present understanding, that in 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 *Cs A* induced renal toxicity and graft rejection, is not possible to make only on the basis of serum creatinine levels, since this parameter increases to an equal extent in both conditions.

If the process damages the entire tubular system, both transferases would be excreted into the urine, like in the case of ATN where the serum creatinine level increases in both types of ARF, but the better correlation was found with α*GST.* In obstructive ARF enzymuria is ordinarelly present from the previously obstructed kidney.The use of urinary levels of *GSTs* for diagnostic purposes may rise certain problems like a variations in the normal value between different individuals or in the same person at different times.Wishing to extend this studies by examining patients with renal pathology not connected to transplantation, we have analyzed two groups of patients with ARF, the first group consisted by 15 cases with global tubular injury (ischaemic ATN) and the second group composed by 7 patients with postrenal, obstructive acute renal insufficiency.

Just as expected, the global ATN affects the both tubular structures (proximal and distal) with significant α/πGSTs-enzymuria. However, the acute renal obstruction provokes slight αGST urinary excretion with extensive and persistent π*GST*-enzymuria.The persistency of elevated πGST into the urine in a few patients with obstructive ARF, may suggest perhaps a more serious initial lesion and/or persistency of other factors like partial obstruction with or without parenchymal infection and medullar necrosis, some of which have not been recognized and considered.Then, the last statement and explanation not opposes the accepted approach that "both increases and decreases in the urinary levels of GST*s* occur rapidly". Furthermore, the increased enzymuria often preceedes (few days) the rise of other functional parameters e.g. the level of serum creatinine. On the other hand, the abscence of GST*s* into the urine in an obstructive process may signify a previous chronic fibrosclerotic kidney lesions of the distal tubules and collecting ducts, before the attack of obstructive ARF, with previously diminished tubular cells reserves of GST*s* (especially πGST)[4]. But, in the cases of chronic heavy metals exposure (like *Cd,Pb,Hg*-intoxication in accumulator industry workers or psychotic patients treated with Li-carbonate*)* many years ago, the urinary GST*s* activity may be 5 - 10 times (especially αGST) upwards of normal limits, even if the toxic agents ceased their activity 5 - 10 years prior to measurement.

Renal infarction as a partial cortical renal necrosis with disseminated intravascular coagulation, or as a medullar necrotic obstructive lesion, may be a further explanation for a massive urinary GST*s* elimination (100 fold increase of both enzymes in the urine), in cases with ARF. The restricted number of analyzed patients in the present study, makes heavy the acceptance of proposed conclusions, then, the further clinical trials are needed to point out more precisely the diagnostic role of GSTs in a variety of clinical conditions associated with consecutive tubular lesion.

Conclusions: ARF is associated with elevated urine levels of GSTs. While the ischaemic ARF has been characterized with predominantely αGST urinary excretion (proximal tubular lesion), acute obstructive kidney insufficiency presents mainly πGST urinary elimination (distal and collecting tubuls injury). Finally, the evolution of urinary GSTs excretion may be valuable tool, differentiating the type and persistency of ARF.

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