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Gamma-Glutamyl Transpeptidase as the Marker of Kidney Graft Function

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Gamma-glutamyl transpeptidase (GGT) is a glycoprotein of the external surface of various cell types. The activity of GGT has been observed in cells and tissues with secretory activity, such as the proximal tubular cells in kidneys. GGT also plays an important pro-oxidant role, stimulating the generation of hydroxyl radicals, and increases membrane lipid peroxidation.
Objectives. In this study we examined whether the monitoring of GGT activity in urine may be a prognostic factor of kidney allograft function. The study enrolled 107 Caucasian renal transplant recipients.
Material and Methods. Urine samples were collected for GGT and creatinine analysis on the 1st day after transplantation, and then in the 3rd and 12th month.
Results. Higher urine GGT activity in the 3rd month after transplantation was associated with significantly higher risk of graft failure (HR = 1.063 per each U/g creatinine; 95%Cl:1.004-1.127; p = 0.037) in the Cox proportional hazards model. Moreover, there were positive correlations between urine GGT and the grade of interstitial fibrosis (Rs = 0.64, p = 0.01) and tubular atrophy (Rs = 0.54, p = 0.056) in specimens collected in the 3rd month after transplantation.
Conclusions. Our results suggest that higher urine GGT activity in the 3rd month after transplantation may be a prognostic factor of graft failure (**Adv Clin Exp Med 2014, 23, 6, 947–952**).

Key words: gamma-glutamyl transpeptidase, kidney, graft.

Gamma-glutamyl transpeptidase (GGT) is an enzyme found widely in tissues with secretory activity from various organisms [1]. This enzyme plays the important role in the metabolism of glutathione (GSH) [2]. Serum GGT activity is often increased in liver diseases, especially in subjects using alcohol [3]. GGT catalyzes the degradation of GSH into glutamate and glutamyl-amino acids, which belong to the reactive thiol compounds with high physiological activity. The main function of these amino acids is the reduction of ferric iron Fe^{3+} into ferrous Fe^{2+} under physiological conditions [4]. This process is associated with generation of reactive oxygen species (ROS) and initiation of oxidative reactions [5, 6]. The highest GGT activity in kidneys was detected on the outer surface of the microvillus membrane in the renal proximal tubule [7, 8]. The activation of GGT in the proximal tubule may enhance oxidative stress leading to kidney damage. In this study we examined whether the monitoring of GGT activity in urine may be a prognostic factor of kidney allograft function.

Material and Methods

The study enrolled 107 Caucasian renal transplant recipients (61.7% males, 38.3% females, mean age 45.56 ± 14.34 years, transplantation performed between 2006 and 2008). The patients were observed in the Clinical Department of Nephrology, Transplantology, and Internal Medicine of the Pomeranian Medical University in Szczecin, Poland. The average period of observation was 30 months (from 0.5 to 60 months). The subjects were selected randomly from among deceased-donor renal transplant recipients. The characteristics of the studied patients are presented in Table 1. The causes of renal failure were: chronic glomerulonephritis (26.17%), autosomal dominant polycystic kidney disease (16.82%), diabetes (13.08%), inflammatory diseases (9.35%), hypertension (7.48%) and other (9.35%). The cause of renal failure remained unknown in 17.75% of the studied patients. The majority of patients (78%) needed hemodialysis before the transplantation, and the rest of the studied subjects were undergoing peritoneal dialysis (22%).

Urine samples were collected for GGT and creatinine analysis on the 1st day after transplantation, and then in the 3rd and 12th month. Among subjects who did not produce urine because of delayed graft function (DGF), the first sample was collected when the amount of urine reached 500 mL per day – not later than in the 3rd day after transplantation. These samples were found to correspond to the urine samples collected on the 1st day after transplantation from patients without DGF and they were analyzed together. The collected samples were centrifuged at 4000 rpm for 10 min and urine, without the sediment, was stored at –80°C until the time of analysis. The activity of GGT was measured by colorimetric method using L-γ-glutamyl-3-carboxy-4-nitroanilide as a substrate (Pointe Scientific, Poland). The 5-amino-2-nitrobenzoate which was released in the reaction was evaluated by measuring the absorbance of the sample compared to the blank sample at a wavelength of λ = 405 nm. The concentration of creatinine in the urine was assessed in a reaction with picric acid, after 50-fold dilution of the urine. The activity of GGT and creatinine concentration in urine was evaluated and GGT activity was calculated in relation to creatinine concentration. Moreover, standard urinalysis was performed and blood samples were collected from all patients to assess serum creatinine and urea, glomerular filtration rate (GFR), blood count and the concentrations of calcineurin inhibitors at the time points of urine sample collection and during standard follow-up visits. In addition, the donor data was analyzed – the time of cold ischemia (CIT), human leukocyte antigen (HLA) mismatches, panel reactive antibody (PRA) and DGF occurrence. DGF was defined as the need for dialysis within the first week after transplantation. Moreover, part of the observed patients underwent protocol biopsies in the 3rd and 12th month after transplantation. Among individuals with delayed graft function, kidney biopsy was performed during the first 2 weeks after transplantation.

Table 1. Clinical characteristics of the studied renal transplant recipients.

Characteristic	N	Mean ± SD
Time of observation [months]	107	29.82 ± 21.59
Age [years]	107	45.56 ± 14.34
Dialysis before Tx [months]	82	24.32 ± 17.74
Residual diuresis [mL]	75	676.7 ± 849.1
Weight [kg]	78	70.72 ± 12.81
CIT [hrs]	87	22.42 ± 9.64
Mismatch A	85	1.24 ± 0.7
Mismatch B	85	1.31 ± 0.67
Mismatch DR	85	0.72 ± 0.63
HLA points	81	12.43 ± 4.2
PRA [%]	76	3.17 ± 6.64

SD – standard deviation, Tx – transplantation, CIT – cold ischemia time, PRA – panel reactive antibody.

Statistical Analysis

We used STATISTICA 9 software (StatSoft, Tulsa, USA) for analysis. As a Shapiro-Wilk's test showed that the distributions of GGT activity were significantly different from normal ($p < 0.05$), we used a non-parametric Mann-Whitney's *U* test, Wilcoxon signed-rank test and Spearman's rank correlation test for the statistical analysis. The Cox proportional hazards model was used for the analysis of graft failure to calculate hazard ratio (HR) and its 95% confidence interval (95%CI). P-value < 0.05 was considered as statistically significant.

Results

The values of urine GGT activity are shown in Table 2. The activity significantly decreased after the first day, but there was no significant difference between the 3rd and 12th months after transplantation.

Table 2. Urine GGT activity on the 1st day, in 3rd and 12th month after renal transplantation

Time after transplantation	N	GGT activity [IU/g creatinine] mean ± SD
1 day	73	35.32 ± 42.8
3 months	56	12.34 ± 10.4
12 months	49	15.07 ± 15.4

SD – standard deviation, GGT – gamma-glutamyl transpeptidase.
Significance of differences (Wilcoxon signed-rank test):
3 months vs. 1 day: p = 0.000005
12 months vs. 1 day: p = 0.0022
12 months vs. 3 months: p = 0.21.

Urine GGT Activity on the 1st Day After Transplantation

There was no statistically significant association between urine GGT activity on the 1st day after transplantation and early graft function (occurrence of DGF), but a trend to higher GGT in recipients with DGF (n = 19) when compared to those without DGF (n = 54) was observed (56.1 ± 71.6 vs. 28.0 ± 23.3 IU/g creatinine, p = 0.067, Mann-Whitney test).

Urine GGT Activity in the 3rd Month After Transplantation

Higher urine GGT activity in the 3rd month after transplantation was significantly associated with the development of graft failure in a univariate Cox model (HR = 1.063 per each IU/g creatinine; 95%Cl: 1.004–1.127; p = 0.037), while no such association was found for the other GGT measurement time points.

Moreover, there was a statistically significant positive correlation between GGT activity and the grade of interstitial fibrosis in specimens collected 3 months after transplantation (p = 0.01) and a borderline positive correlation with tubular atrophy (p = 0.05) (Table 3, Fig. 1 and 2).

Urine GGT Activity in the 12th Month after Transplantation

There was a statistically significant (p = 0.02) positive correlation between GGT activity in the 12th month after transplantation and the grade of

Table 3. Correlation of GGT urine activity with changes in renal biopsies

Time of GGT measurement after transplantation	Renal biopsy Banff parameter and time of measurement	N	Rs	p
1 day	I1	8	0.62	0.09
	CI1	8	–0.41	0.31
	CT1	8	–0.07	0.85
	T1	8	0.41	0.31
	ATN1	8	0.08	0.85
3 months	I3	13	0.33	0.26
	CI3	13	0.64	0.01
	CT3	13	0.54	0.05
	T3	13	0.27	0.37
12 months	CI3	14	0.6	0.02
	I12	20	0.01	0.98
	CI12	20	0.11	0.62
	CT12	20	0.22	0.34
	T12	20	–0.08	0.71
	ATN12	18	–0.02	0.90

I – interstitial infiltration, CT – tubular atrophy, CI – interstitial fibrosis, T – tubulitis, ATN – acute tubular necrosis, Rs – Spearman rank correlation coefficient.

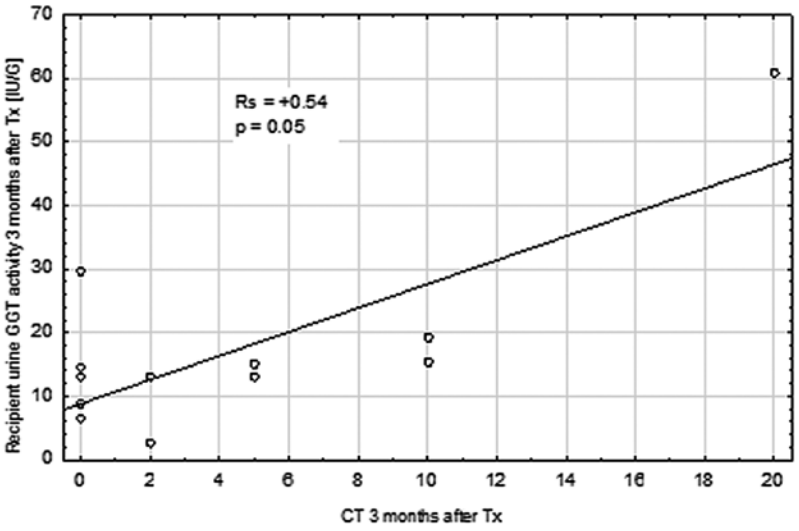

Fig.1. Correlation between recipient urine GGT activity and Banff CT parameter (tubular atrophy) 3 months after transplantation

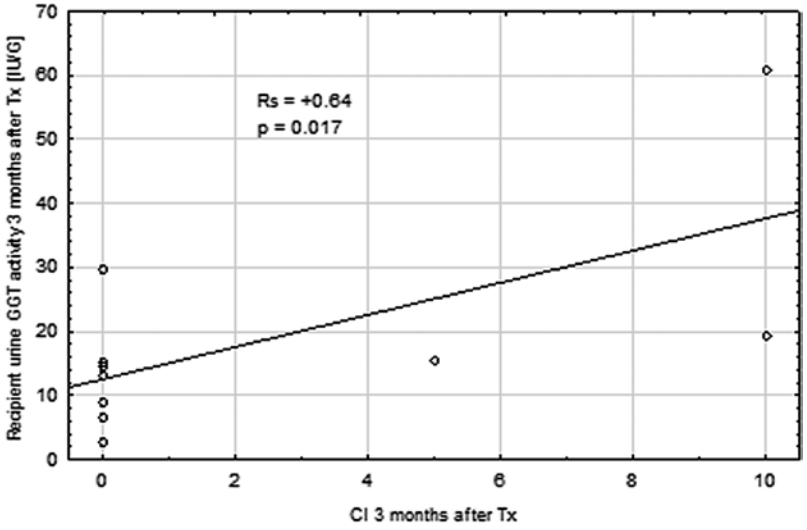

Fig. 2. Correlation between recipient urine GGT activity and Banff CI parameter (interstitial fibrosis) 3 months after transplantation

interstitial fibrosis in specimens collected 3 months after transplantation (Table 3).

Discussion

In our study, we analyzed GGT urine activity in patients after renal transplantation on the 1st day as well as in the 3rd and 12th months after transplantation. We have not observed a statistically significant correlation between GGT urine activity and early graft function (delayed graft function). The urine GGT activity on the 1st day after transplantation may be dependent on various clinical factors such as ischemia-reperfusion, warm and cold ischemia and dialysis. Therefore, the urine GGT activity on the 1st day after transplantation is not a prognostic factor of graft function. We observed a correlation between urine GGT activity in the 3rd month after transplantation and changes in renal biopsies. There were statistically significant positive correlations between urine GGT activity and the grade of interstitial fibrosis and tubular atrophy in specimens collected in the 3rd month after transplantation.

Glutamyl transpeptidase has the critical function of breaking down extracellular glutathione into amino acids that subsequently can be taken up by the cell [9]. GGT is secreted as a propeptide. [10]. GGT has been found to be an essential component of the metabolic activation of a series of halogenated alkenes and quinones to nephrotoxins [9, 10]. These compounds form glutathione-conjugates that are hydrolyzed to cysteinylglycine-conjugates by GGT on the surface of the renal proximal tubule cells. The conjugates are further cleaved to cysteine-conjugates by aminopeptidase which is also on the surface of the cell. The cysteine-conjugates are taken up into the cell and converted to a reactive thiol.

Studies have revealed that ischemia induced swelling and morphological changes in the proximal tubules of kidneys in rats [12]. This process was associated with increased GGT activity and decreased synthesis of GSH [13, 14]. Aviacin (an inhibitor of GGT) prevented the increase of GGT activity

induced by ischemia and protected the ischemic organs and tissues against lipid peroxidation, free radical generation and morphological changes. [15].

The above findings suggest that the increased GGT activity may be associated with the ischemic damage of tissues such as the tubules of kidneys. Therefore, the inhibition of GGT activity may protect the kidneys from ischemia–reperfusion injury [16, 17]. Yamamoto et al. studied the effects of treatment with a GGT inhibitor on ischemia-reperfusion induced renal injury in nephrectomized rats [18]. Treatment with a GGT inhibitor before ischemia decreased the ischemia-reperfusion induced kidney injury. These authors observed increased activity of GGT and increased synthesis of superoxide and malondialdehyde in the kidneys after reperfusion. Moreover, a GGT inhibitor prevented the histopathological changes in kidneys. The above changes were prevented by the administration of a GGT inhibitor, which additionally increased the level of glutathione. These results demonstrate that GGT may be involved in ischemia-reperfusion induced ischemic acute kidney injury [18].

Our results suggest that the urine GGT activity in the 3rd month after transplantation positively correlated with the grade of interstitial fibrosis and tubular atrophy.

Currently, the main cause of renal graft loss is glomerulosclerosis, interstitial fibrosis and tubular atrophy [19]. Nankivell et al. evaluated prospective protocol kidney-transplant biopsies from recipients taken regularly up to 10 years after transplantation for evidence of glomerular injury [20]. The first grade of Banff chronic interstitial fibrosis was detected in 94.2% of recipients in 3 months after transplantation. These authors suggest that the first 3 months are crucial in the development of allograft nephropathy and subsequent graft loss.

Our results suggest that higher urine GGT activity in the 3rd month after transplantation may be a prognostic factor of graft failure.

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