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Gammaglutamyltransferase in ESRD as a predictor of all-cause and cardiovascular mortality: another facet of oxidative stress burden

Maurizio Postorino[1,2], Carmen Marino[1,2], Giovanni Tripepi[1,2] and Carmine Zoccali[1,2]

[1]*Dialysis and Transplantation and CNR-IBIM Clinical Epidemiology and Pathophysiology of Renal Diseases and Hypertension, Reggio Calabria, Italy*

The enzyme γ-glutamyltransferase (GGT) is an established marker of liver function and alcohol consumption and represents the major factor responsible for the extra-cellular catabolism of the main antioxidant in mammalian cells, Glutathione. ESRD is a condition characterized by a high risk of death and cardiovascular (CV) complications and with a high prevalence of liver disease but the link between GGT and clinical outcomes has never been studied in this population. We tested the predictive power of GGT for overall and cardiovascular mortality in a cohort study in 584 ESRD patients. Over a 4 years follow up 194 patients died. GGT was higher in non-survivors (median 25 UI/l, interquartile range 16–45 UI/l) than in survivors (22, 15–33 UI/l) ($P = 0.006$). On univariate Cox regression analysis plasma GGT predicted both all-cause [HR (10 UI/l increase): 1.04, 95% CI: 1.01–1.06, $P = 0.006$] and cardiovascular mortality [HR: 1.03, 95% CI: 1.00–1.05, $P = 0.04$]. These relationships held true in multivariate Cox regression analyses [HR: 1.06, 95% CI: 1.03–1.10 ($P < 0.001$) and 1.05, 95% CI: 1.01–1.10, $P = 0.01$] adjusting for liver disease as well as Framingham risk factors and non traditional risk factors including C reactive Protein (CRP). High GGT in ESRD patients is a strong, independent risk marker for all cause and cardiovascular death. The predictive power of GGT for these outcomes likely reflects the involvement of this enzyme in oxidative stress mechanisms.

Kidney International (2008) **74** (Suppl 111), S64–S66; doi:10.1038/ki.2008.515

KEYWORDS: γ-glutamyltransferase; risk; ESRD; dialysis; cardiovascular; CRP

Correspondence: *Carmine Zoccali, CNR-IBIM, presso Euroline, Via Vallone Petrara 57, Reggio Calabria 89124, Italy. E-mail: carmine.zoccali@tin.it*

[2]On behalf of the Working Group of the Calabrian Registry of Dialysis and Transplantation.

γ-Glutamyltransferase (GGT) is a cell-surface enzyme that plays a major role in the extracellular catabolism of glutathione (GSH), a main antioxidant.[1] GGT is virtually ubiquitary, and the liver is the major source of circulating levels of this enzyme. Liver disease, high alcohol intake, dyslipidemia, and hyperglicemia may alter plasma GGT.[1] Perhaps, as an expression of its role on GSH metabolism, high GGT level has been associated with the occurrence of cardiovascular (CV) disease and death in various populations,[2–5] an effect that appears to be independent of liver disease as well as of traditional classical and novel risk factors cardiac risk factors such as C-reactive protein (CRP).[5] It can be noted that the involvement of GGT in atherosclerosis is suggested by the observation that it localizes in atherosclerotic plaques.[6]

ESRD is a condition characterized by an exceedingly high risk of death and CV complications. In ESRD, oxidative stress is considered as the final pathway mediating CV damage triggered by a variety of traditional and non-traditional risk factors.[7] Among these risk factors, malnutrition and inflammation appear to be prominent, from an epidemiological point of view, and of particular relevance in the generation of the high oxidative stress of ESRD patients.[7] On the other hand, the prevalence of liver disease is increased in ESRD and underlies a high death risk in this population.[8] The fact that GGT is a marker of both oxidative stress and liver disease makes this protein an interesting biomarker in ESRD. However, the link between GGT and clinical outcomes has never been studied in this disease. With this background in mind, we performed a prospective study to investigate the relationship between circulating GGT all-cause and CV mortality in a large cohort of ESRD patients maintained on chronic hemodialysis treatment.

RESULTS

GGT was above the upper limit of the normal range (cutoff: 75 UI/l) in 50 out of 584 ESRD patients (9%). High GGT was directly associated with male sex ($P = 0.03$), HCV positivity ($P < 0.001$), dialysis vintage ($P = 0.04$), and serum CRP ($r = 0.15$, $P < 0.001$), and there was a tendency for a direct link between GGT and background CV complications

($P = 0.08$). Furthermore, GGT was inversely related with diastolic pressure ($r = -0.10$, $P = 0.02$) and serum phosphate ($r = -0.16$, $P < 0.001$). In a multiple linear regression model including all univariate correlates of GGT, only HCV positivity ($\beta = 0.17$, $P < 0.001$), phosphate ($\beta = -0.15$, $P < 0.001$), CRP ($\beta = 0.11$, $P = 0.01$), and male sex ($\beta = 0.09$, $P = 0.03$) maintained an independent relationship with circulating levels of GGT.

GGT and all-cause and CV mortality

During follow-up (average 2.4 years, range 0.1–3.9), 194 patients died; of these, 121 (62%) died because of CV causes. GGT was significantly higher ($P = 0.006$) in patients who died (median: 25 UI/l, IQR: 16–45 UI/l) than in those who survived (median: 22 UI/l, IQR: 15–33 UI/l), and the same association emerged in patients who developed CV events (median: 26 UI/l; IQR: 17–47 UI/l) as compared with those who remained free of CV events (median: 22 UI/l; IQR: 15–34 UI/l) ($P = 0.01$). Accordingly, on univariate Cox regression analysis, plasma GGT seemed to be strongly related to all-cause (hazard ratio (HR) (10 UI/l increase in GGT): 1.04, 95% CI: 1.01–1.06, $P = 0.006$) and CV mortality (HR: 1.03, 95% CI: 1.00–1.05, $P = 0.04$). In multivariate Cox regression analyses (Table 1), the predictive power of GGT for all-cause death (HR: 1.05, 95% CI: 1.02–1.08, $P = 0.003$) and CV death (HR: 1.05, 95% CI: 1.01–1.10, $P = 0.01$) was largely independent of liver disease as well as of other potential confounders, including age, sex, diabetes, diastolic pressure, and serum CRP.

Table 1 | Multivariate Cox regression analysis modeling the association of GGT with all-cause and cardiovascular mortality

	Units on increase	HR and 95% CI	*P*-value
All-cause mortality			
Age	1 year	1.07 (1.05–1.08)	<0.001
Diabetes	0=no; 1=yes	2.44 (1.69–3.51)	<0.001
CRP	10 mg/l	1.14 (1.05–1.23)	0.001
GGT	10 UI/l	1.05 (1.02–1.08)	0.003
BMI	1 kg/m^2	0.95 (0.91–0.99)	0.008
Pulse pressure	1 mm Hg	1.01 (1.00–1.02)	0.02
Duration of RDT	1 month	1.01 (1.00–1.02)	0.03
HbsAg positivity	0=no; 1=yes	0.69 (0.21–2.28)	0.54
HCV positivity	0=no; 1=yes	1.12 (0.77–1.62)	0.56
Male sex	0=female; 1=male	1.05 (0.78–1.41)	0.77
Triglycerides	1 mg/100 ml	1.00 (0.99–1.01)	0.93
Calcium × phosphate	1 mg^2/100 ml^2	1.00 (0.99–1.01)	0.89
CV mortality			
Age	1 year	1.08 (1.06–1.10)	<0.001
Diabetes	0=no; 1=yes	2.78 (1.78–4.35)	<0.001
CRP	10 mg/l	1.17 (1.06–1.29)	0.001
BMI	1 kg/m^2	0.93 (0.88–0.98)	0.009
GGT	10 UI/l	1.05 (1.01–1.10)	0.01
Pulse pressure	1 mm Hg	1.01 (1.00–1.03)	0.01
Calcium × phosphate	1 mg^2/100 ml^2	1.01 (1.00–1.02)	0.02
HCV positivity	0=no; 1=yes	1.37 (0.86–2.16)	0.18
Male sex	0=female; 1=male	1.17 (0.80–1.72)	0.41
Triglycerides	1 mg/100 ml	1.00 (0.99–1.01)	0.56
Duration of RDT	1 month	1.00 (0.99–1.01)	0.78
HbsAg positivity	0=no; 1=yes	0.83 (0.19–3.64)	0.81

BMI, body mass index; CI, confidence interval; CRP, C reactive protein; GGT, γ-glutamyltransferase; HCV, hepatitis C virus; HbsAg, hepatitis B surface antigen; HR, hazard ratio; RDT, regular dialysis treatment.
Covariates in the multivariate model were selected because of their relation with study outcomes with $P < 0.05$ at univariate analysis. Forcing background CV complications into the models did not materially change the strength of the relationship between GGT with all-cause (HR (10 UI/l increase in GGT): 1.05, 95% CI: 1.02–1.08, *P*=0.002) and CV mortality (HR (10 UI/l increase in GGT): 1.06, 95% CI: 1.01–1.10, *P*=0.008).

DISCUSSION

This study in ESRD patients shows that GGT predicts all-cause and CV death independent of liver disease, traditional and non-traditional risk factors in this population. The results are compatible with the hypothesis that the link between GGT and adverse clinical outcomes depends on the fundamental role of this enzyme in oxidative stress mechanism(s).

Liver disease, particularly HCV hepatitis, is frequent in ESRD[9] and associated with greater morbidity and mortality.[8] Serum GGT levels are increased in hepatitis B surface antigen-positive and/or anti-HCV-positive patients, and about 20% of dialysis patients with chronic viral hepatitis display GGT levels above the upper limit of the normal range.[10] Alcohol dependency, although rare in ESRD,[11] also appears associated with a high death risk. In line with observations in dialysis centers in western European countries,[9] in our cohort 11% of patients had anti-HCV antibodies and only 2% of patients were HBV-positive. Remarkably, in this study, GGT coherently predicted all-cause and CV mortality in analyses adjusting for HBV and HCV positivity. Thus, the prognostic power of high GGT level in ESRD is largely independent of liver disease, an observation corroborated by the fact that the estimate of death risk conveyed by this enzyme was almost identical before and after statistical adjustment for hepatotropic viruses positivity.

GGT is considered as a marker of hepatic steatosis in patients with metabolic syndrome.[5] However, it is unlikely that this applies to ESRD patients. Indeed, in keeping with earlier studies,[7] our data once again confirm that a low rather than a high BMI predicts mortality in ESRD. We believe that the strong relationship between GGT and adverse clinical outcomes in ESRD depends on the fundamental role of this enzyme in oxidative processes. Perhaps, because it is transported by lipoproteins flowing into atherosclerotic plaques, GGT is well represented in atherosclerotic lesions.[6] One of the products of extracellular GSH hydrolysis produced by GGT is cysteinylglycine, which can generate superoxide anion radicals through its interaction with free iron,[12] a phenomenon that can promote atherogenesis through LDL oxidation. However, high GGT level should not be seen solely as a pro-oxidant enzyme. GSH metabolism is initiated by GGT, which is then completed by membrane dipeptidases. The released amino acids, particularly cysteine, serve as essential substrates for intracellular GSH and protein synthesis. Genetic GGT deficiency in the GGT_{enu1} mouse impairs GSH metabolism and transport and, results in systemic glutathionemia, glutathionuria, low GSH levels in

the liver, and oxidant stress in the kidney.[13] Exposure to an inhaled oxidant (NO_2) induces GGT gene expression within the lung epithelium and GGT protein accumulation, implying an active role for GGT-mediated GSH metabolism at the epithelial surface of the lung.[14] Therefore, increased GGT synthesis and high plasma levels of this enzyme may be a counter-regulatory response aimed at ensuring adequate GSH supply in situations of high oxidative stress. Oxidative stress is pervasive in ESRD.[7] However, perhaps because no single oxidative stress marker adequately reflects the multifaceted biochemical consequences of oxidative stress,[15] only few studies in ESRD patients could document the deleterious effects of this major pathway to organ damage on mortality and CV complications.[7] Thus, independent of the nature of the GGT link with oxidative processes, GGT represents an interesting oxidative stress marker that can be used in clinical studies in ESRD patients.

MATERIALS AND METHODS

The analyses described in this study are based on the dialysis registry of a southern Italian region (Calabrian REgistry of DIalysis and Transplantation, CREDIT) affiliated with the Italian and the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA) Registries. This region has a resident population of 2.1 million and an ESRD prevalence of 655 patients per million population.

CREDIT includes the data of all patients with ESRD treated in 36 dialysis units of the same region and includes standard data collected by the ERA-EDTA registry as well as by detailed information on comorbidities and other data. Of 36 centers, 24 (67%) participated in this study.

A total of 584 patients were enrolled in this study. These patients represented 51% of the whole hemodialysis population on treatment in the Calabria region in 2003 ($n = 1143$) and were representative of the whole regional dialysis population for age, sex, diabetes, comorbidities, and all major clinical variables included in CREDIT.

Demographic, clinical, and anthropometric data of all patients treated with extracorporeal dialysis in participating centers were obtained during 2003. Plasma levels of GGT as well as of alanine and aspartate aminotransferase (ALT and AST) levels, hepatitis B surface antigen and hepatitis C virus (HCV) status, serum lipids, calcium, phosphate, and hemoglobin were tested by standard methods. Serum levels of CRP were measured by a high sensitivity nephelometric method (DADE-Behring, Scoppito, L'Aquila, Italy). All measurements were obtained midweek before dialysis.

All patients were followed up until December 2006 or until censored (study end, kidney transplantation, or lost to observation).

Statistical analysis

Data are reported as mean ± s.d., median, and interquartile range (IQR) or as percent frequency, as appropriate, and comparisons among groups were made by *P*-value for trend. Variables with a positively skewed distribution were log-transformed (lg10) before the correlation study (Pearson's product moment correlation coefficient (r) and *P*-value.

The independent factors explaining the variability in GGT in the study population were investigated by univariate and multiple linear regression analyses, and the predictive power of GGT for all-cause and CV mortality was investigated by univariate and multiple Cox regression analyses. All calculations were performed using a standard statistical package (SPSS for Windows Version 9.0.1, 11 March 1999, Chicago, IL, USA).

DISCLOSURE

The authors have declared no financial interests.

ACKNOWLEDGMENTS

This work is the result of a joint effort with the following members of the Working Group of the Calabrian Registry of Dialysis and Transplantation: S. Chiarella, V. Martire, O. Santoro, G. Scida, G. Ascoli, A.F. Minisci, E. D'Anello, A. Reina, T. Urso, A. Foscaldi, A. Mellace, G. Mazza, V. Rocchetti, P. Procopio, R. Roberti, F. D'Agostino, C. Franco, I. Maimone, M.L. Mannino, A. Sellaro, E. Garofalo, C. Fornaciari, D. Plutino, M. Rovito, F.S. Fabiano, N. Lucà, G. Rizzuto, M. Senatore, E. Barreca, O. Marzolla, D. Galati, A. Pugliese, F. Grandinetti, M. Gullo, V. Bruzzese, M. De Gaudio, M. Bovino, and G. Alati.

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