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Serum gamma-glutamyltransferase fractions in Myotonic Dystrophy type I: Differences with healthy subjects and patients with liver disease

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ABSTRACT

Objectives: Elevation of serum gamma-glutamyltransferase (GGT), in absence of a clinically significant liver damage, is often found in Myotonic Dystrophy type-1 (DM1).
In this study we investigated if a specific GGT fraction pattern is present in DM1.

Designs and methods: We compared total and fractional GGT values (b-, m-, s-, f-GGT) among patients with DM1 or liver disease (LD) and healthy subjects (HS).

Results: The increase of GGT in DM1 and LD, vs HS, was mainly due to s-GGT (median: 32.7; 66.7; and 7.9 U/L, respectively), and b-GGT (8.5; 18.9; and 2.1 U/L). The subset of DM1 patients matched with HS with corresponding serum GGT showed higher b-GGT (6.0 vs 4.2 U/L).

Conclusions: DM1 patients with normal total GGT values showed an alteration of the production and release in the blood of GGT fractions. Since increased s-GGT is also found in LD, a sub-clinical liver damage likely occurs in DM1 subjects apparently free of liver disease.

Introduction

Elevation of serum gamma-glutamyltransferase (GGT), a sensitive biomarker of liver disease, is frequently found in Myotonic Dystrophy type-1 (DM1) [1], nevertheless, most patients fail to show a clinically significant liver damage. GGT values of DM1 patients are rather associated with biomarkers of oxidative damage in vivo such as advanced oxidation protein products (AOPP) and ferric reducing ability of plasma (FRAP) [2]; in fact GGT is known for catalyzing free radical formation and oxidative damage in vitro by releasing the potent iron reducing dipeptide cysteinylglycine [3]. Clarifying the mechanisms of GGT increase in DM1 patients might help understanding the pathogenesis of the disease, thus improving its management.

Circulating GGT is a heterogeneous entity consisting of soluble protein and several molecular complexes, provided with distinct physico-chemical properties [4]. Recently, we set up a method to separate these complexes on the basis of the molecular weight [5]. In human blood, obtained from healthy subjects, this procedure allowed the identification of four GGT fractions: big-GGT (b-GGT), medium-GGT (m-GGT), small-GGT (s-GGT) and free-GGT (f-GGT), with molecular weight ranging between >2000 kDa (b-GGT) and 70 kDa (f-GGT).

The aim of the present study was to establish if a specific disease-associated form of GGT is present in DM1 patients. Thus fractional GGT pattern of DM1 patients has been compared with that of healthy subjects and patients with liver disease."

Materials and methods

Study population

Twenty-nine consecutive patients with DM1, (18 males, age 43 ± 12), were compared with 29 patients with miscellaneous liver disease

Abbreviations: DM1, Myotonic Dystrophy type I; GGT, Gamma-glutamyltransferase; b-GGT, big-GGT; m-GGT, medium-GGT; s-GGT, small-GGT; f-GGT, free-GGT; HS, healthy subjects; LD, liver disease patients.

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(LD, 17 males, age 56 ± 15; 15 subjects had cholestatic diseases while 14 showed steatosis not related to alcohol abuse) and 29 healthy subjects (HS, 18 males, age 45 ± 13), of corresponding age and gender. HS were selected in a database of 256 blood donors [6]. Liver disease in DM1 patients was excluded on the basis of clinical examination, ultrasound tomography, and determination of standard clinical laboratory tests for liver disease. Two DM1 patients had been subjected to surgery for gallstones, 5 and 8 years before being included in the study.

A second group of controls ($n = 17$) was established by matching each DM1 patient with a healthy subject with corresponding gender and serum GGT activity. In this case, since the 12 MD patients with higher serum GGT did not find a match out of the database of 256 blood donors, only 17 individuals were included.

The Institutional Ethics Committee approved the study and all subjects gave informed consent.

GGT fraction analysis

Fasting plasma samples (0.02 mL) were analysed as previously described [5] by a HPLC System Gold apparatus (Beckman 126) equipped with a spectrofluorometric detector (821-FP, Jasco Europe). Separation and quantification of fractional GGT was obtained by gel filtration chromatography (Superose 6 HR 10/300 GL column; GE Healthcare Europe) followed by post-column injection of the fluorescent substrate for GGT, gamma-glutamyl-7-amido-4-methylcoumarin (gGluAMC). Enzymatic reaction, in the presence of gGluAMC 0.030 mmol/L and glycylglycine 4.5 mmol/L, proceeded for 4.5 min in a reaction coil (PFA, 2.6 mL) kept at the 37 °C in a water bath. The fluorescence detector operating at excitation/emission wavelengths of 380/440 nm detected the AMC signal; the intensity of the fluorescence signal was expressed in arbitrary fluorescence units (f.u.). Under this reaction conditions, area under curve is proportional to GGT activity. Total area, between 10 and 25 mL elution volume, and fractional GGT area was calculated by a MatLab program (Version 7 MathWorks, Inc.) to resolve overlapping peaks; the curve fitting was conducted with a nonlinear least-squares minimization algorithm using four exponentially modified Gaussian (EMG) curves. The reaction was calibrated analysing plasma samples with known total GGT activity (standards). The slope of the calibration curve was used to convert total and fractional GGT area to U/L [5,6]. The sum of fractional GGT activity represents on average the 99% of total GGT activity.

Statistical analysis was conducted by ANOVA, (Kruskal–Wallis test followed by Dunn's multiple comparison test), except that for matched data, where the Wilcoxon matched pair test was adopted.

Results

GGT fraction pattern in DM1 and LD patients

To establish if a specific disease-associated form of GGT is present in DM1 patients, we compared the results with fractional GGT values of 29 healthy subjects (HS) and in the same number of patients affected by liver disease (LD), matched for age and gender.

In all DM1 and LD patients, independently from total GGT values, we found the same four GGT fractions (b-, m-, s-, f-GGT) described in the population of blood donors [6], from which we selected the 29 HS (Fig. 1).

As expected, DM1 patients showed higher total GGT levels (median, 25th–75th percentile: 54, 26–108 U/L) in comparison with HS (25, 19–34 U/L), but lower than in LD group (99, 67–181 U/L; Table 1). f-GGT was the predominant fraction in HS, but not in DM1 and LD. In fact, in the latter groups the increase of GGT was exclusively due to higher levels of the fractions b-GGT, m-GGT and s-GGT (Table 1).

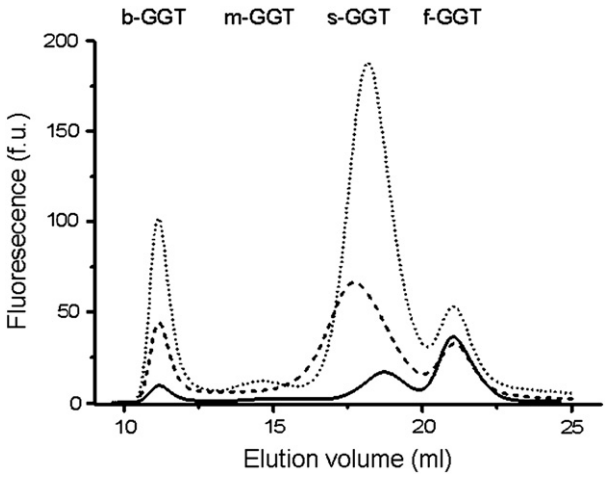

Fig. 1. Representative elution profile of fractional GGT activity for a healthy subject (continuous line: b-GGT 2.5 U/L, m-GGT 1.7 U/L, s-GGT 7.9 U/L, f-GGT 13.9 U/L), a DM1 patients (dashed line: b-GGT 9.6 U/L, m-GGT 4.7 U/L, s-GGT 35.0 U/L, f-GGT 9.9 U/L) and a patient with liver disease (dotted line: b-GGT 18.9 U/L, m-GGT 6.9 U/L, s-GGT 70.9 U/L, f-GGT 14.4 U/L). These profiles are representative of the median fractional GGT distribution in the three groups.

In comparison with LD group, DM1 patients showed lower values of total GGT, s-GGT and f-GGT, while the fraction b-GGT and m-GGT were not significantly different.

Among DM1 patients, seventeen showed total GGT levels (range 14–60 U/L) within the reference values. Thus we selected a group of healthy subjects with corresponding total GGT values to compare fractional GGT pattern and we found that also these DM1 patients had an altered pattern of fractional GGT. In fact, DM1 patients showed higher b-GGT values than healthy subjects (6.0 U/L vs 4.2 U/L, $p < 0.05$), but lower f-GGT (11.0 U/L vs 12.4 U/L, $p < 0.05$).

Discussion

The main finding of this study is that the increase of serum GGT in DM1 is due to s-GGT, and to a minor extent to b-GGT, while the most abundant GGT fraction in HS is f-GGT. Since increased s-GGT is also found in LD, these results support the hypothesis that a sub-clinical liver damage occurs in DM1 subjects apparently free of liver disease. Even the subset of DM1 patients with lower total GGT values, compared to healthy subjects matched for total GGT activity, showed an altered pattern of GGT fractions with significantly higher b-GGT and lower f-GGT. Thus an early impairment of b-GGT production and release in the blood might precede liver damage.

The pathophysiological significance of s-GGT and b-GGT might be different. In fact, in healthy subjects both fractions are significantly

Table 1
Total and fractional GGT values (U/L) in healthy subjects (HS), patients with miscellaneous liver disease (LD) and patients with myotonic Dystrophy type I (DM1).

	HS	DM1 p vs HS	LD p vs HS	p DM1 vs LD
Total GGT	24.7 (18.6–34.6)	54.3 (25.8–107.7) <0.01	98.9 (67.2–181.2) <0.001	<0.01
b-GGT	2.1 (1.5–5.0)	8.5 (4.9–29.4) <0.001	18.9 (13.5–28.4) <0.001	n.s.
m-GGT	1.0 (0.5–1.5)	3.1 (1.6–7.8) <0.001	5.3 (3.2–7.8) <0.001	n.s.
s-GGT	7.9 (5.7–14.8)	32.7 (10.0–53.0) <0.001	66.7 (36.1–118.5) <0.001	<0.01
f-GGT	12.2 (10.6–13.7)	11.1 (10.2–16.5) n.s.	15.6 (13.3–20.0) <0.01	<0.01

Data are median (25th–75th percentile).
Statistical analysis: Kruskal–Wallis test followed by Dunn's multiple comparison test.

associated with AST and ALT transaminases, but b-GGT is also independently associated with the inflammatory biomarker C-reactive protein and several cardiovascular risk factors [7]. Thus, s-GGT might be a marker of liver damage, as suggested by its paramount increase in LD subgroup, while b-GGT potentially correlates with tissue damage in organs other than liver.

The idea that all serum GGT originates from the liver derives from previous studies in subjects with cholestasis and very high values of serum GGT. The increase of lipophylic high molecular weight forms of GGT led to hypothesize that circulating GGT might derive from the absorption of GGT over circulating lipoproteins [4], such as LP-x which is specifically produced during cholestasis [4]. Nevertheless the mechanism of GGT release in blood and its tissue origin has not been established for certain and might be different for each fraction. We recently found that human cells of non-hepatic origin, including normal bronchial epithelium, are able to release in serum-free culture medium a GGT form corresponding to b-GGT [8]. This suggests that, at least in part, b-GGT might derive from the shedding of cell membranes endowed with high GGT activity. Since all tissues display cell membrane GGT activity, even higher than the liver [9], the increase of b-GGT might correspond to the enhanced GGT release from diseased tissues, other than the liver, but also to a decreased clearance of the fraction. The idea of a differential pathogenetic significance for b-GGT is supported also by our finding that it is the only fraction found within human atherosclerotic plaques [10], where it is co-localised with oxidised LDL and CD68+ foam cells [3,11].

For this reason, if considering the prooxidant role of GGT [12] and the connection between serum GGT and oxidative damage in vivo in DM1 [2], identifying the source and the metabolic fate of s-GGT and b-GGT might contribute to the understanding of the pathogenesis of DM1, and in particular of the role played by oxidative damage in the complex phenotype of this disease.

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