Plasma γ-glutamyl Transferase Activity Predicts Homocysteine Concentration in a Large Cohort of Unselected Outpatients

Giuseppe Lippi¹, Gian Luca Salvagno¹, Giovanni Targher², Martina Montagnana¹ and Gian Cesare Guidi¹

Abstract

Objective  To evaluate the association between γ-glutamyl transferase (GGT) and homocysteine in plasma.
Methods  Combined results of GGT, total homocysteine, vitamin B12, folate and creatinine levels were analyzed in outpatients referred for laboratory testing over the past 5 years.
Results  Cumulative results for homocysteine, GGT, B12, creatinine, folate could be retrieved for 449 outpatients >35 years old over the study period. The concentration of homocysteine significantly increased among tertiles of GGT activity. In multivariable linear regression analysis, GGT activity was independently associated with homocysteine concentration, independent of age, gender, folate, vitamin B12 and serum creatinine levels.
Discussion  Plasma GGT activity might be a useful means to predict homocysteine concentration in the general population.

Key words: cardiovascular risk, γ-glutamyl transferase, folic acid, homocysteine


Introduction

Recent population-based epidemiological studies have convincingly shown that serum γ-glutamyl-transferase (GGT) activity is associated with many cardiovascular risk factors and predicts new-onset type 2 diabetes, hypertension, stroke and myocardial infarction (1, 2). In particular, Sakuta et al have shown that serum GGT activity is associated with total cholesterol, triglyceride, fasting plasma glucose, total homocysteine and systolic blood pressure independent of potential confounders, such as cigarette smoking, alcohol consumption and body mass index (3). Accordingly, we have recently confirmed that serum GGT activity is independently associated with a more atherogenic lipid profile in the general population (4). The clinical implications of these findings may be noteworthy, in that people with low-normal GGT (e.g., <40 U/L) would no longer be considered at high risk of developing cardiovascular disease according to the traditional strategy of risk assessment. Although the mechanism has not been elucidated, GGT elevation occasionally anticipates the occurrence of diabetes and cardiovascular disease. In this homology, it has further been suggested that GGT might also predict homocysteine elevation (3), which is a potential risk factor for thrombosis and cardiovascular disease (5). However, although the association between GGT, plasma lipids and lipoproteins has been consistently investigated, to date little information is available on the relationship between GGT and homocysteine concentrations. In fact, the potential biological relationship, or the simple association, between GGT and homocysteine, might deserve scrutiny to help prevent or better predict the cardiovascular risk in patients with hyperhomocysteinemia.

¹Division of Clinical Chemistry, University of Verona Medical School, Verona, Italy and ²Division of Endocrinology and Metabolic Diseases, University of Verona Medical School, Verona, Italy

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Correspondence to Dr. Gian Luca Salvagno, gianluca.salvagno@univr.it gsalvagno77@yahoo.it
Table 1. Baseline Characteristics of the Study Participants (n=449) according to Tertiles of Serum γ-Glutamyl Transferase (GGT) Activity

<table>
<thead>
<tr>
<th>GGT tertiles (U/L)</th>
<th>Unadjusted P values for trend</th>
<th>P values for trend adjusted for age and sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥20-39.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>172</td>
<td>158</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>31%</td>
<td>65%</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 (49-55)</td>
<td>58 (56-61)</td>
</tr>
<tr>
<td>Homocysteine (μmol/L)</td>
<td>13.5 (12.0-15.1)</td>
<td>14.5 (13.1-16.0)</td>
</tr>
<tr>
<td>% pts ≥15 μmol/L</td>
<td>30%</td>
<td>39%</td>
</tr>
<tr>
<td>Folate (nmol/L)</td>
<td>7.2 (5.3-9.0)</td>
<td>7.6 (4.8-10.4)</td>
</tr>
<tr>
<td>Vitamin B₁₂ (pmol/L)</td>
<td>477 (102-2118)</td>
<td>468 (110-2008)</td>
</tr>
</tbody>
</table>

Methods

To further investigate this potentially meaningful relationship, we performed a retrospective analysis on the database of the Laboratory Information System of the Clinical Chemistry Laboratory at the Verona University Hospital to retrieve results of serum GGT, homocysteine and, vitamin B₁₂ (B₁₂) and folic acid tests, which have been performed on the whole cohort of outpatients consecutively referred by general practitioners for routine blood testing over the past 5 years (April 2002-April 2007). Venous blood from fasting outpatients was routinely collected in the morning. GGT was assayed by enzymatic procedures on a Roche/Hitachi Modular System (Roche Diagnostics GmbH, Mannheim, Germany). Total homocysteine which refers to the sum of homocysteine, homocysteine, and homocysteine-cysteine mixed disulfide, free and protein bound was determined on EDTA plasma by high-performance liquid chromatography (HPLC) with fluorescent detection, according to Araki and Sako (6). Folate and B₁₂ concentrations were assayed by an automated chemiluminescence method (Chiron Diagnostics, East Walpole, MA, USA). Significance of differences and frequency distribution of values were assessed by the Kruskal-Wallis test (for continuous variables) and the chi-squared test (for categorical variables), respectively. Serum creatinine was also assayed on a Roche/Hitachi Modular System P (Roche Diagnostics GmbH), by creatinine Jaffe, rate blanked and compensated assay. The independence of the association between total homocysteine and GGT was tested by multivariable linear regression analysis. Skewed variables (homocysteine, folate, B₁₂, creatinine) were logarithmically transformed to improve normality prior to this analysis. In the fully adjusted multivariable regression model, plasma homocysteine was entered as the dependent variable, whereas age, gender, GGT, folic acid and serum creatinine were included as covariates. Statistical analyses were performed using the statistical package SPSS-version 12. Data are presented as means (±95% confidence intervals) or percentages and the level of statistical significance was always set at p<0.05.

Results

Cumulative results for homocysteine, GGT, B₁₂, folate and serum creatinine could be retrieved for 449 outpatients >35 years old over the 5-year period (M/F=236/213; mean age 56 years, 95% CI: 53-59 years). As shown in Table 1, the concentration of homocysteine, but not that of folate and B₁₂, significantly increased among the tertiles of serum GGT activity. Similarly, the frequency of subjects with homocysteine values exceeding 15 μmol/L, which has been identified as the cutoff value for cardiovascular risk by most clinical studies (7), increased steadily across the spectrum of GGT thresholds, from 30% to 49%. These results remained essentially unchanged even after adjusting for sex and age. Accordingly, in multivariable linear regression analysis, total homocysteine concentration was associated with GGT (standardized beta coefficient = 0.057; p<0.001) independently of age, sex, B₁₂, folate and serum creatinine.

Discussion

Although the definitive pathogenesis is still unclear, it has been highlighted that GGT activity may be regarded as a marker of cardiovascular risk or oxidative stress rather than
a mere indicator of excessive alcohol consumption or obesity (1, 2). In particular, the association between GGT activity and a variety of traditional cardiovascular risk factors has been hypothesized to explain, at least partially, the reported relationships between increased concentrations of this liver enzyme and cardiovascular disease (1-4). However, to our knowledge, the information available on the relationship between GGT and homocysteine is scarce. In the only one published study on Japanese adult men, Sakuta et al found that total homocysteine was significantly correlated with increased concentrations of this liver enzyme and cardiovascular disease (1-4). However, to our knowledge, the information available on the relationship between GGT and homocysteine is scarce. In the only one published study on Japanese adult men, Sakuta et al found that total homocysteine was significantly correlated with increased concentrations of this liver enzyme and cardiovascular disease (1-4). Overall, the findings of the present study confirm the observations by Sakuta et al, though we extended the analysis on both genders. Moreover, we also ruled out any potential confounding influence from the concentration of folic acid and B12, which are recognized as major determinants of plasma homocysteine concentrations (8). We could not evaluate the methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphisms in our study population. However, it has been recently been reported that frequency of this genotype (homozygote or heterozygote) is not statistically different between healthy controls and patients with nonalcoholic steatohepatitis or nonalcoholic fatty liver (9), which are the main determinants of increased GGT values in the general population.

The possible underlying biological mechanisms of the observed association between GGT activity and homocysteine concentration are still poorly known. It has been suggested that a possible molecular mechanism underlying the homocysteine toxicity may involve its metabolic conversion to homocysteine thiolactone, which can react with lysine residues in proteins, damaging their structure and impairing their physiological activities. The extent of homocysteine thiolactone synthesis and protein homocysteinylatination in human vascular endothelial cells is principally dependent by levels of homocysteine, methionine, folate and high-density lipoprotein that are linked to both vascular disease and efficiency of the hydrolytical detoxification (10).

Conditions that increase serum GGT lead to increased free radical production and the threat of glutathione depletion. The products of the GGT reaction may themselves lead to increased free radical production (11, 12). Therefore, enhanced GGT activity in plasma, which primarily reflects an increased oxidative stress, would be associated with less effective mechanisms of homocysteine detoxification, so increasing its toxicity. In this perspective, the measurement of serum GGT activity concentrations in the general population might be helpful to predict not only the cardiovascular risk associated with traditional risk factors, such as impaired glucose tolerance, hypertension and plasma lipids, but also that associated with an increased homocysteine concentration. Although this investigation was focused on the biological association between GGT and homocysteine in plasma, we are aware that the lack of information on past cardiovascular events or cardiovascular risk factors in this large cohort of unselected outpatients might be a limitation in the study.

References


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