Is Serum Gamma-Glutamyltransferase a Biomarker of Xenobiotics, Which Are Conjugated by Glutathione?

Duk-Hee Lee, Myron D. Gross, Michael W. Steffes, David R. Jacobs Jr

A meta-analysis of 10 prospective cohort studies, including our own, reported that serum γ-glutamyltransferase (GGT) predicted incident vascular events. This meta-analysis posed one important, but unsolved question: why does serum GGT predict incident vascular events? In addition to vascular events, serum GGT predicts various important clinical outcomes including type 2 diabetes, metabolic syndrome, and renal problems.

Serum GGT has traditionally been used as a marker of alcohol consumption; however, alcohol consumption cannot explain the disease relationships with serum GGT because these associations have been observed among nondrinkers as well as drinkers. The other prevailing interpretations are that serum GGT predicts clinical outcomes as a marker of nonalcoholic fatty liver disease (NAFLD) or oxidative stress. Although both of these explanations have some merit, each also has problems.

Although not clearly stated in many of the reports, most associations of serum GGT with clinical outcomes were dose-response relations within the normal range of serum GGT; we submit that this aspect of the association is critical to interpretation. First, even though NAFLD may partially explain the associations among subjects with a relatively high serum GGT activity and NAFLD can present even among subjects with low normal serum GGT, NAFLD is unlikely to explain the graded associations with clinical outcomes which were observed among subjects with serum GGT in the low part of its normal range, less than 20 or 30 U/L. However, it is worthwhile to note that serum GGT may be a predictor of the future risk of NAFLD. In a prospective study by our group, serum GGT within its normal range strongly predicted the future risk of chronic elevation of serum alanine aminotransferase (ALT), but serum ALT within its normal range did not predict the future risk of chronic elevation of serum GGT. The most common reason for elevated serum ALT, especially among nondrinkers, is NAFLD. We refer to this issue below in relation to xenobiotics.

Several years ago our research group proposed that serum GGT within its normal range should be regarded as a marker of oxidative stress, as seen from two contrary perspectives. As a primary function, cellular GGT metabolizes extracellular glutathione (GSH) to allow precursor amino acids to be assimilated and reused for intracellular GSH synthesis. Thus, cellular GGT can be seen as an antioxidant enzyme because increased cellular GGT favors the supply for intracellular GSH, the most important intracellular antioxidant. On the other hand, cellular GGT can be involved in the generation of oxidative stress in the presence of transition metals. Even though the direct relation between cellular GGT and serum GGT under the physiological condition is unclear, serum GGT has been assumed to reflect these properties of cellular GGT.

However, the concept of serum GGT as primarily either an antioxidant or a pro-oxidant marker presents a challenge in understanding the GGT and disease relationships. As an antioxidant marker, even though the initial increase of GGT may be compensatory to depleted GSH because of oxidative stress, serum GGT would be expected to decrease the risk of clinical outcomes because the parallel increase in cellular GGT would lead to increased intracellular GSH. However, epidemiological studies contradict this, consistently reporting serum GGT within its normal range predicts increasing risk of various clinical outcomes. From the opposite perspective, it is difficult to believe that pro-oxident effects of GGT would occur across the low normal range of serum GGT in vivo, considering that GGT is indispensable in metabolizing GSH, an essential component in the cell. Although we do not exclude the possibility that GGT itself can be involved in generating oxidative stress in vivo, this too seems unlikely to be a significant factor across the low normal range of GGT.

A potentially interesting interpretation subsumes GGT as a biomarker of exposure to xenobiotics. We have recently reported that some environmental pollutants such as lead, cadmium, dioxins, or organochlorine pesticides are positively and monotonically related to serum GGT in the general population without any occupational exposure. Interestingly, the associations between environmental pollutants and serum ALT, a more liver specific enzyme and more commonly used as a marker of NAFLD than serum GGT, were opposite to those of serum GGT, suggesting that the associations of serum GGT may not be related to liver toxicity.

Indeed, GSH has a very important function in conjugating xenobiotics to facilitate their excretion. In general, metabolism of xenobiotics consists of phase I and phase II reactions. In phase I, xenobiotics are generally converted to more polar, hydroxylated derivatives. In phase II, these derivatives are conjugated with molecules such as glucuronic acid, sulfate, or GSH. This renders them more water-soluble, facilitating excretion in the urine or bile. GSH conjugates...
synthesized in the cells need be mobilized into the extracellular space for further metabolism. Just as cellular GGT is indispensable for metabolism of extracellular GSH, GGT is also needed to prepare extracellular GSH conjugates for further metabolism to mercapturic acid, the final form of GSH conjugates found in urine or bile. In this way, higher serum GGT plausibly reflects increased cellular GGT activity to metabolize extracellular GSH conjugates. Thus, serum GGT increases with increasing exposure to xenobiotics which need to be conjugated to GSH. For example, the expression of cellular GGT is the most common phenotypic marker of preneoplastic foci in the livers of carcinogen-treated rats.

The concept of serum GGT as a marker of xenobiotic exposure complements our earlier assertion that serum GGT relates to oxidative stress. Various exogenous xenobiotics can directly increase free radical production, increasing endogenous ROS by-products. In addition, endogenous by-products of reactive oxygen species (ROS) are also conjugated by GSH. Furthermore, consumption of intracellular GSH through conjugation itself can increase intracellular oxidative stress. All these mechanisms suggest that the exposure to xenobiotics is associated with oxidative stress. However, metabolizing extracellular GSH without xenobiotics has different implications than metabolizing GSH conjugates with xenobiotics. The former concept implies increasing intracellular GSH by providing precursor amino acids for intracellular GSH synthesis, but the latter concept implies using intracellular GSH for conjugating xenobiotics and results in its depletion.

GGT conjugates substances which are hydrophilic and contain an electrophilic atom. Conjugation can occur even without the presence of the catalytic activity of GSH transferase. Various electrophilic xenobiotics can accept an electron pair to form a covalent bond and react with nucleophilic sites of biomolecules. Thus, electrophilic xenobiotics may be highly dangerous to the cell, because they are able to bind to proteins and nucleic acids and thereby disturb metabolic networks and cause mutations. At the cellular level, GSH conjugation is primarily a defense mechanism against toxic electrophilic compounds, although some GSH conjugation can be involved in bioactivation of xenobiotics. However, from a broad perspective, the absolute amount of GSH conjugates may reflect a cumulative internal exposure dose of toxic electrophilic compounds which are conjugated with GSH. This idea about serum GGT led us to focus on Persistent Organic Pollutants (POPs) as being among possible xenobiotics that can explain some epidemiological findings on serum GGT.

In fact, exposure of liver cells to some xenobiotics has led to morphological changes, such as proliferation of smooth endoplasmic reticulum and fatty change in hepatocytes, both of which are early biomarkers of liver degeneration. These considerations are consistent with the observation that serum GGT as a marker of xenobiotics predicts the future risk of NAFLD which we observed in the previous study; but unlike the currently prevailing interpretation, is not a marker of the concurrent presence of NAFLD.

Obesity is accepted as a strong risk factor for NAFLD. POPs link to obesity in that they bioaccumulate in adipose tissue, and, as endocrine disruptors, may interact with obesity in disturbing both glucose and lipid metabolism. Therefore obese persons who have bioaccumulated a considerable amount of POPs may have a higher risk of NAFLD and related conditions than obese persons without POPs. The fact that low risk for diabetes was observed in the obese persons who had low POPs or GGT supports this view.

This concept can also provide an interpretation of the finding of weak associations between serum GGT and vascular diseases among older subjects. It is well known that hepatic metabolizing capacity of xenobiotics decreases with age, a phenomenon that reduces the ability to clear xenobiotics. At older compared to younger ages, the production of glutathione conjugates may be lower for a fixed level of exposure to xenobiotics. Because cellular and therefore serum GGT increase proportional to the amount of glutathione conjugates, not the amount of xenobiotics themselves, cellular or serum GGT activities in older people may not reflect the extent of exposure to xenobiotics as well as in younger people.

Taken together, based on both well-known biochemistry about the metabolism of xenobiotics and empirical epidemiological evidence, we suggest that serum GGT within its normal range may predict various clinical outcomes as a marker of internal dose of xenobiotics which are conjugated with GSH. This concept is a comprehensive one which modifies currently prevailing interpretations of serum GGT as a marker of NAFLD or oxidative stress. We think that traditional epidemiological studies may have limited capacity to directly confirm our hypothesis. A molecular epidemiological approach which measures cellular GGT, serum GGT, GSH conjugates, a variety of xenobiotics, and clinical outcomes may be helpful to further study our hypothesis. In addition, it would be helpful to study the association between cellular GGT, serum GGT, and GSH conjugates in cell models.

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References


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