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Serum gamma-glutamyltransferase within its normal range predicts a chronic elevation of alanine aminotransferase: A four year follow-up study

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Abstract

Background: Previous epidemiological and experimental studies support the concept that serum gamma-glutamyltransferase (GGT) activity within its normal range is related to oxidative stress. Since oxidative stress plays a crucial role in the pathogenesis of various liver diseases, serum GGT may predict development of liver damage.

Methods: A total of 6,523 healthy male workers with normal alanine aminotransferase (ALT, <35 U/l) in a steel manufacturing company were followed for four years. Liver damage was defined as a chronic elevation of serum ALT (both 2001 and 2002).

Results: After adjusting for age, body mass index, alcohol consumption, cigarette smoking, exercise, and baseline value of ALT, in comparison with the group whose GGT level was <10 U/l, the adjusted relative risks for elevated ALT level among those with GGT levels 10–19, 20–29, 30–39, and over 40 U/l was 1.0, 2.5, 4.7, 7.4, and 12.0, respectively (P for trend <0.01). More importantly, this association was similarly observed even among non-drinkers; the corresponding relative risks were 1.0, 1.8, 3.8, 5.6, and 6.2 (P for trend <0.01). However baseline ALT did not predict abnormal GGT level four years later.

Conclusion: Serum GGT levels within normal range predict incidence of chronic elevation of ALT. Oxidative stress might explain this relationship.

Keywords: *Gamma glutamyltransferase, oxidative stress, alanine aminotransferase, liver damage*

Although highly elevated serum gamma-glutamyltransferase (GGT) is a well-known enzyme that marks alcohol consumption, alcoholic liver diseases, or cholestasis[1,2], our previous studies[3–6] have shown that serum GGT within its normal range is an early and sensitive marker closely related to oxidative stress. Consistent with our findings, experimental studies[7–9] have reported that GGT has a central role in glutathione homeostasis by initiating the breakdown of extracellular glutathione (GSH), a critical antioxidant defense for the cell. Paradoxically, there is evidence that, under physiological conditions, GGT is directly involved in the generation of reactive oxygen species, especially in the presence of iron or other transition metals[10–13].

Oxidative stress plays a crucial role in the induction and in the progression of various liver diseases including viral hepatitis, alcoholic hepatitis,

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non-alcoholic steatohepatitis (NASH), liver fibrosis, liver cirrhosis, or hepatocellular carcinoma[14–16]. If GGT is a marker of oxidative stress within its normal range, it might predict development of liver diseases. NASH might be of particular interest because serum GGT within its normal range has shown a strong graded relationship with incident diabetes[5,17], and NASH is also frequently associated with type 2 diabetes[18–20].

Therefore, we performed a prospective study to test the hypothesis that GGT within its normal range is a predictor of future liver damage. In this study, we used an elevation of serum alanine aminotransferase (ALT) activity above normal on at least two occasions, over a span of at least six months, as a surrogate for liver damage[21]. The most likely histological diagnosis of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients is non-alcoholic fatty liver disease[22].

Materials and methods

Study population

The data analyzed were from periodic worksite health examinations at one large steel company in Korea. Throughout 1998, a health check-up was performed between 9.00 a.m. and noon in a health care center located in the factory. Male workers between 20 and 55 years of age without elevated ALT ($\geq$35 U/l), diagnosed liver diseases (including liver cirrhosis, viral or alcoholic hepatitis, and so on), and hepatitis B antigen were eligible for follow-up in this study. Of the 7,404 men who met these criteria, 6,605 men (89.2 percent follow-up rate) were reexamined in both 2001 and 2002. An additional 82 subjects were deleted due to missing value of variables, leaving 6,523 for analysis. No specific informed consent for this study was obtained. Data are analyzed pursuant to the Korean health regulation pertaining to factories, which states that the factory physician has an obligation to analyze health examination data to educate workers.

Measurements

Information on lifestyle factors including alcohol consumption, cigarette smoking, and exercise were obtained primarily by self-reported questionnaires. Body mass index (BMI) was calculated as weight (kg) divided by weight squared (m^2), both of which were measured at the time of the health examination. Venous blood samples were obtained from an antecubital vein after a 12 h overnight fast. Serum GGT and ALT concentrations (normal range 0–50 U/l for serum GGT; $<$35 U/l for serum ALT) were measured at 37°C with an automatic analyzer (Hitachi 7170, Japan). The serum samples were kept at 4°C and analyzed within 48 h of collection.

Statistical analysis

In this study, the definition of a chronically elevated serum ALT was abnormal level of ALT ($\geq$35 U/l) in both 2001 and 2002. Five categories of baseline GGT level (0–9, 10–19, 20–29, 30–39, $\geq$40 U/l) were analyzed using multiple logistic regression analysis, omitting individuals with baseline ALT $\geq$ 35 U/l. Covariates were the baseline values of age (years), body mass index (kg/m^2), cigarette smoking (pack-years), smoking status (current, ex, and never), alcohol consumption (g/week), exercise (frequency/week), and ALT (U/l). A stratified analysis by status of alcohol consumption was also performed. In addition, we performed a parallel analysis to see if baseline serum ALT level predicts a chronic elevation of serum GGT ($\geq$50 U/l) in both 2001 and 2002, omitting individuals with baseline GGT $\geq$ 50 U/l. Other restrictions on study subjects were the same as in the analyses in which incident ALT elevation was the dependent variable. The SAS statistical program, version 8.0, was used in all analyses, the p values quoted are two-sided, and those p values <0.05 are regarded as statistically significant.

Results

At baseline, there were clear positive or negative dose-response relationships with serum GGT level among all listed variables in Table I. Subjects with high serum GGT level, mostly within the normal GGT range, tended to be older, drink more alcohol, smoke more, exercise less, and have higher BMI. Follow-up characteristic also showed similar associations, except exercise. Similar associations were observed in both non-drinkers and drinkers.

During the four-year period, incidence of elevated ALT level was 5.1% (334 of the 6523 workers). Baseline serum GGT level within its normal range predicted the incidence of elevated ALT level in a strong graded dose-response manner. In comparison with the group whose GGT level was $<$10 U/l, the adjusted relative risks for elevated ALT level among those with GGT levels 10–19, 20–29, 30–39, and over 40 U/l were 1.0, 2.5, 4.7, 7.4, and 12.0, respectively (P for trend <0.01, Table II). The dose-response association was apparent in both non-drinkers and drinkers (Table III). The corresponding figures among non-drinkers were 1.0, 1.8, 3.8, 5.6, and 6.2 (P for trend <0.01) and those among drinkers were 1.0, 3.5, 6.3, 10.0, and 16.6 (P for trend <0.01).

On the other hand, without adjusting for baseline GGT, baseline ALT also appeared to predict the incidence of elevated GGT level (Model 1 in Table IV). However, the association completely

Table I. Baseline and follow-up characteristics according to categories of serum GGT of the cohort in 1998 among 6523 male workers.

		Categories of serum GGT (U/l) in 1998					
		<10 (*n*=648)	10–19 (*n*=3960)	20–29 (*n*=1326)	30–39 (*n*=377)	≥40* (*n*=232)	P_{trend}
Baseline characteristics in 1998	Arithmetic mean ± SD						
	Age (years)	35.4 ± 6.0	37.2 ± 6.2	38.7 ± 5.9	40.1 ± 5.8	41.2 ± 5.0	<0.01
	Alcohol consumption (g/week)	59.9 ± 76.5	92.7 ± 96.2	133.4 ± 118.2	149.9 ± 110.5	177.2 ± 122.3	<0.01
	Cigarette smoking (pack-years)	5.3 ± 6.5	7.5 ± 8.0	9.6 ± 8.2	11.1 ± 8.6	12.1 ± 8.0	<0.01
	BMI (kg/m^2)	21.4 ± 2.0	22.4 ± 2.2	23.4 ± 2.1	23.9 ± 2.2	23.8 ± 2.3	<0.01
	Serum ALT (U/l)	18.8 ± 5.1	22.5 ± 5.7	26.3 ± 5.5	27.8 ± 4.7	27.8 ± 4.7	<0.01
	Proportion						
	Regular exercise (%)	32.7	25.0	25.3	26.9	21.1	<0.01
Follow-up characteristic in 2002	Arithmetic mean ± SD						
	Alcohol consumption (g/week)	76.9 ± 80.0	108.6 ± 101.8	148.8 ± 123.7	155.4 ± 111.6	172.7 ± 111.4	<0.01
	Cigarette smoking (pack-years)	6.7 ± 6.3	8.2 ± 7.3	9.8 ± 7.7	11.0 ± 8.4	11.9 ± 8.5	<0.01
	BMI (kg/m^2)	22.2 ± 2.1	23.1 ± 2.2	23.9 ± 2.2	24.4 ± 2.4	24.3 ± 2.3	<0.01
	Serum ALT (U/l)	20.6 ± 7.9	24.5 ± 13.1	28.4 ± 12.4	31.4 ± 15.2	31.5 ± 11.5	<0.01
	Proportion						
	Regular exercise (%)	34.7	32.9	35.2	33.1	36.8	0.93

* 64 had GGT ≥ 50 U/l.

Table II. Adjusted relative risks (95% confidence interval, CI) for incidence of elevated ALT during the follow-up period by GGT in 1998 among 6,523 male workers (baseline ALT ≥ 35 U/l omitted).

	GGT level (U/l) in 1998 [normal range 0–50]					
	0–9	10–19	20–29	30–39	40–	P_{trend}
Cases/N at risk (%)	5/651 (0.8%)	126/3963 (3.2%)	112/1325 (8.5%)	51/375 (13.6%)	40/209 (19.1%)	<0.01
Adjusted RR (95%, CI)						
Model 1*	1.0	4.5 (1.8–10.9)	13.1 (5.3–32.3)	23.3 (9.2–59.1)	36.3 (14.0–94.1)	<0.01
Model 2†	1.0	2.6 (1.1–6.4)	4.8 (1.9–12.1)	7.6 (2.9–19.6)	12.0 (4.5–31.7)	<0.01
Model 3‡	1.0	2.5 (1.0–6.3)	4.7 (1.9–11.9)	7.4 (2.8–19.5)	12.0 (4.5–32.3)	<0.01

* Adjusted for age. † Additional adjustment for baseline ALT. ‡ Additional adjustment for the baseline values of age, alcohol consumption, smoking, exercise, and BMI.

Table III. Adjusted relative risks (95% confidence interval, CI) for incidence of elevated ALT stratified by status of alcohol consumption during the follow-up period by GGT in 1998 among 6523 male workers.

		GGT level (U/l) in 1998 [normal range 0–50]					
		0–9	10–19	20–29	30–39	40–	P_{trend}
Non-drinker	Cases/N at risk (%)	3/270 (1.1%)	37/1024 (3.6%)	22/195 (11.3%)	6/39 (15.4%)	2/14 (14.3%)	
	Adjusted RR (95% CI)						
	Model 1*	1.0	3.7 (1.1–12.0)	13.4 (3.9–45.9)	20.7 (4.8–88.6)	21.0 (3.1–142.1)	<0.01
	Model 2†	1.0	2.1 (0.6–7.1)	4.9 (1.3–17.7)	7.4 (1.6–33.4)	7.6 (1.1–53.9)	<0.01
	Model 3‡	1.0	1.8 (0.5–6.1)	3.8 (1.0–14.2)	5.6 (1.2–26.1)	6.2 (0.9–45.4)	<0.01
Drinker	Cases/N at risk (%)	2/381 (0.5%)	89/2939 (3.0%)	90/1130 (8.0%)	45/336 (13.4%)	38/195 (19.1%)	<0.01
	Adjusted RR (95% CI)						
	Model 1*	1.0	6.2 (1.5–25.2)	17.8 (4.4–72.8)	32.9 (7.9–137.5)	53.1 (12.6–224.5)	<0.01
	Model 2†	1.0	3.6 (0.9–14.6)	6.3 (1.5–26.2)	10.0 (2.4–42.5)	16.4 (3.8–70.3)	<0.01
	Model 3‡	1.0	3.5 (0.9–14.6)	6.3 (1.5–26.2)	10.0 (2.2–42.9)	16.6 (3.8–72.2)	<0.01

* Adjusted for age. † Additional adjustment for baseline ALT. ‡ Additional adjustment for the baseline values of age, alcohol consumption, smoking, exercise, and BMI.

Table IV. Adjusted relative risks (95% confidence interval, CI) for incidence of elevated GGT during the follow-up period by ALT in 1998 among 6,523 male workers (baseline GGT ≥ 50 U/l omitted).

	ALT level (U/l) in 1998 [normal range 0–35]					
	0–9	10–19	20–29	30–39	40–	P_{trend}
Cases/N at risk (%)	0/3 (–)	18/1901 (1.0%)	121/3305 (3.7%)	87/1718 (5.1%)	43/613 (7.0%)	
Adjusted RR (95% CI)						
Model 1*	1.0¶		3.9 (2.4–6.4)	5.4 (3.3–9.1)	7.8(4.5–13.7)	<0.01
Model 2†	1.0¶		1.2 (0.7–2.2)	0.8 (0.4–1.4)	0.6 (0.3–1.1)	<0.01
Model 3‡	1.0¶		1.3 (0.8–2.3)	0.8 (0.5–1.5)	0.6 (0.3–1.2)	<0.01

*Adjusted for age. †Additional adjustment for baseline GGT. ‡Additional adjustment for the baseline values of age, alcohol consumption, smoking, exercise, and BMI. ¶The two lowest ALT categories were combined.

disappeared after adjusting for baseline GGT (Model 2 in Table IV). These analyses imply that baseline ALT was correlated with the baseline level of GGT, but not with its consistent change into the abnormal range during follow-up.

Discussion

This study demonstrated a strong, positive, dose-response relationship for serum GGT levels at baseline, mostly within the normal range, with incidence of chronic elevation of ALT with four years of follow-up. This association was similarly observed in both non-drinkers and drinkers. However, serum ALT levels at baseline did not predict the incidence of elevated GGT level.

Oxidative stress plays a crucial role in the induction and in the progression of various liver diseases[14–16]. Oxidative stress initiates and regulates the transcription and activation of a large series of other mediators in all liver cells, which culminate in common mechanisms of liver damage: apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression, and regeneration[14]. The prevalence and the persistence of one or more of these aspects may influence the occurrence of the different types of liver diseases[14].

A series of our previous studies[3–6]. has consistently shown that serum GGT within its normal range may be an early and sensitive marker that is related with oxidative stress. First, baseline serum GGT within its normal range predicted in a dose-response manner C-reactive protein, a marker of inflammation, and F2-isoprostanes, a marker of oxidative damage to arachidonic acid[3]. Second, dietary heme iron positively predicted future serum GGT level[4], free iron is a critical catalyst in generating reactive oxygen species[23]. Third, most dietary antioxidants and serum antioxidants inversely predicted future serum GGT level[4,5].

Even though serum GGT is known as a liver enzyme, this enzyme is widely distributed in human body, especially in kidney and liver, and is frequently localized to the plasma membrane with its active site directed into the extracellular space[24]. At a cellular level, GGT has been known to play an important role in antioxidant defense systems[7–9]. GGT catalyzes the initial step in the degradation of extracellular GSH, thereby providing a supply of constituent amino acids for uptake and reutilization in intracellular GSH synthesis. GSH plays an important role in protecting cells against oxidants that are produced during normal metabolism. If oxidative stress increases, then so will the requirement for reduced glutathione. Conversely, if glutathione is not available then the cells will be more vulnerable to development of oxidative stress. The importance of GGT in maintaining adequate levels of intracellular glutathione under normal conditions has been demonstrated across a number of cell types, tissues, or organs[25]. Paradoxically, recent experimental studies[10–13] clearly indicate that GGT may also be involved in the generation of reactive oxygen species. This effect of GGT seems to occur when GGT is induced in the presence of iron or other transition metals. Specifically, cysteinylglycine, which is one of the products of the GGT action, has a strong ability to reduce Fe^{3+} to Fe^{2+}, which again promotes generation of free radical species such as lipid peroxides. Therefore, taken all previous findings together, our finding can be interpreted as an association between serum GGT as an early and sensitive marker of oxidative stress, including that related to liver tissue, and development of liver damage.

Generally, patients with abnormal liver function tests (LFT) over more than six months are considered to have a chronically elevated LFT[21]. The most common etiologies for chronically elevated LFT are alcohol use, viral hepatitis, and non-alcoholic steatohepatitis[21,22]. In our study, it was almost impossible to specify causes of the chronic elevation of ALT because we did not have any histological diagnosis. However, as we excluded men with known liver diseases and hepatitis B antigen carriers, it is reasonable to suppose that the majority of men with chronic elevation of ALT might be patients with

alcoholic or non-alcoholic steatohepatitis. As oxidative stress is related with any type of liver diseases[14–16], this kind of limitation might not be a critical issue in interpreting our study.

In conclusion, serum GGT levels within its normal range may predict incidence of chronic elevation of ALT, possibly as a marker of oxidative stress. Since measurement of serum GGT is inexpensive and easy, if our finding is true, it can be very useful in both clinical and epidemiological studies.

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