ELSEVIER

Atherosclerosis 178 (2005) 327–330

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

Association between serum gamma-glutamyltransferase and C-reactive protein

Duk-Hee Lee[a,*], David R. Jacobs Jr.[b,c]

[a] *Department of Preventive Medicine, Division of Preventive Medicine and Health Promotion Research Center, School of Medicine, Kyungpook National University, 101 Dongin-dong, Jung-gu, Daegu 700 422, Korea*

[b] *Department of Epidemiology, School of Public Health, University of Minnesota, Minnesota, USA*

[c] *Department of Nutrition, University of Oslo, Oslo, Norway*

Received 14 April 2004; received in revised form 9 August 2004; accepted 13 August 2004
Available online 12 October 2004

Abstract

A series of epidemiological studies have suggested serum gamma glutamyltransferase (GGT) within its normal range might be an early marker of oxidative stress. Oxidative stress appears to be a key component of many reactions associated with chronic inflammation. Therefore, we examined the cross-sectional association between deciles of serum GGT and concentrations of serum C-reactive protein (CRP), a marker of chronic inflammation, among 12,110 adult participants in the third U.S. National Health and Nutrition Examination Survey. After adjustment for race, sex, age, cigarette smoking, alcohol intake, and body mass index (BMI), serum concentration of GGT across all deciles was positively associated with serum concentrations of CRP (P for trend < 0.01). For example, adjusted relative risks of serum CRP ≥ 3.0 mg/L by deciles of serum GGT were 1.0, 1.23, 1.40, 1.59, 1.62, 1.61, 2.17, 2.38, 2.45, and 3.41 (P for trend < 0.01). This association was consistently observed among all subgroups; Non-Hispanic White, Non-Hispanic Black, Mexican American, men, women, non-drinkers, drinkers, non-smokers, ex-smokers, current smokers, BMI < 25, BMI 25–29.9, and BMI ≥ 30. The strong association of serum GGT and CRP suggest that further studies on cellular and/or serum GGT might help to elucidate the association between oxidative stress and inflammation.

Keywords: Gamma glutamyltransferase; C-reactive protein; Oxidative stress; Inflammation

1. Introduction

Recently, in an attempt to improve global cardiovascular risk prediction, considerable interest has focused on C-reactive protein (CRP), a marker of inflammation that has been shown in multiple prospective epidemiological studies to predict incident myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death [1,2].

On the other hand, serum gamma glutamyltransferase (GGT) levels within normal range was strongly associated with most cardiovascular disease risk factors and predicted the development of heart disease, hypertension, stroke, and type 2 diabetes [3–9]. In particular, serum GGT level has shown a strong graded relationship with incident diabetes, suggesting a role in the pathogenesis of diabetes [4,5]. Although serum GGT activity has been commonly used as a marker for excessive alcohol consumption or liver diseases [10], neither alcohol consumption nor liver dysfunction likely explained these associations [4,5]. A series of Coronary Artery Risk Development in Young Adults (CARDIA) studies [5,11,12] suggested that oxidative stress might explain these associations because serum GGT within normal range had dose-response relations with serum and/or dietary antioxidant vitamins and markers of oxidative stress such as F2-isoprostanes. Although the relationship between cellular GGT and serum GGT is not known, cellular GGT has been known to play an important role in antioxidant defense systems [13–16]; paradoxically, cellular GGT may also be involved in the generation of reactive oxygen species in the presence of transition metals [17–21].

* Corresponding author. Tel.: +82 53 420 6960; fax: +82 53 425 2447.
E-mail address: lee_dh@knu.ac.kr (D.-H. Lee).

0021-9150/$ – see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.atherosclerosis.2004.08.027

Oxidative stress appears to be a key component of many reactions associated with chronic inflammation [22–24]. Multiple oxidative processes play a critical role in inflammation and act on various intra- and extracellular pathways through specific mediators in conjunction with free radicals that amplify inflammatory reactions at specific sites [22–24]. In this context, the CARDIA finding of serum GGT predicting future concentrations of CRP in a dose-response manner could be of substantial interest. To our knowledge, however, the CARDIA finding [5] is the only report examining the association of serum GGT with CRP concentration. Therefore, we investigated the association between serum GGT and CRP concentration among another group of subjects, namely a representative sample of the US population using the third National Health and Nutrition Examination Survey (NHANES III).

2. Materials and methods

A detailed description of NHANES III can be found elsewhere [25]. Briefly, NHANES III, conducted from 1988 to 1994, was a national probability sample designed to provide national estimates of the health and nutritional status of the US civilian, noninstitutionalized population aged 2 months and older. Of the 18,825 NHANES III participants aged 20 years and older, we excluded 6518 with missing serum GGT or CRP and 288 who were pregnant, leaving 12,110 individuals available for analysis.

The NHANES III data collection included a standardized home interview followed by a detailed physical examination in a mobile evaluation clinic or the participant's home. Information on a wide variety of sociodemographic, medical history, nutritional history (food frequency questionnaire), and family history questions, such as self-reported age, race/ethnicity, gender, history of smoking, alcohol consumption, use of vitamin supplements, and 24 h dietary recall, were obtained during the home interview.

Laboratory methods and quality control procedures are described in detail elsewhere [25]. A venous blood sample was collected and shipped weekly at −20 °C. Serum GGT concentration was assayed using a Hitachi 737 Analyzer (Boehringer-Mannheim Diagnostics, Indianapolis, IN) at White Sands Research Center. Serum CRP was analyzed using latex-enhanced nephelometry. The limit of detection for this assay was 3.0 mg/L; subjects with undetectable levels were assigned a level of 2.1 mg/L.

Serum GGT levels were classified as deciles; cutoff points of deciles of serum GGT were 11, 14, 16, 19, 21, 25, 31, 40, and 62 U/L (normal range: 11–51 U/L for men, 7–33 U/L for women). Serum CRP level was right-skewed, so results were presented as geometric means of serum CRP level across deciles of serum GGT levels. We also presented results using cutpoints of ≥3.0 mg/L to define "detectable" levels and ≥10.0 mg/L to define "highly elevated" levels. Linear and logistic regression models were adjusted for age, race, sex, smoking status (no-smoker, ex-smoker and current smoker), alcohol intake (g/day), and body mass index (BMI) (kg/m^2). In this study, the internal validity was a more important issue than generalization to the total US population, therefore we did not use a specific analytic method to consider the sampling frame of NHANES III.

We repeated the same analyses after stratifying by race (Non-Hispanic White, Non-Hispanic Black, versus Mexican American), sex (men versus women), alcohol consumption status (non-drinkers versus drinkers), smoking status (non-smokers, ex-smokers, versus current smokers), and BMI (<25, 25–29.9, versus ≥30). For the stratified analyses, quintile of serum GGT was used for statistical stability.

For a comparison with serum GGT, we reanalyzed the association between serum alanine aminotransferase (ALT), a more liver-specific enzyme, and serum CRP. Serum ALT levels were also classified as deciles; cutoff points of deciles of serum ALT were 7, 9, 10, 12, 13, 15, 18, 22, and 30 U/L (normal range: <40 U/L for men, <31 U/L for women).

3. Results

The average age of the sample was 48.8 years. The proportions of ethnic groups were 44.0% for non-Hispanic white, 27.9% for non-Hispanic black, 23.5% for Mexican–American, and 4.6% for others. There were more females (52.8%) than males (47.2%) in the sample.

Serum GGT, mostly within normal range, was positively associated with serum CRP in a dose-response manner whatever criteria of serum CRP was used; geometric means, ≥3.0 mg/L, or ≥10.0 mg/L (Table 1). For example, adjusted relative risks of serum CRP ≥ 3.0 mg/L by deciles of serum GGT were 1.0, 1.23, 1.40, 1.59, 1.62, 1.61, 2.17, 2.38, 2.45, and 3.41 (P for trend < 0.01). On the contrary, serum ALT was not positively, if any inversely, associated with serum CRP. Adjusted relative risks of serum CRP ≥ 3.0 mg/L by deciles of serum ALT were 1.0, 0.92, 0.87, 0.75, 0,87, 0.77, 0.74, 0.81, 0.79, 0.86 (P for trend = 0.02).

The strong positive association of serum GGT with CRP was similarly observed among all subgroups; Non-Hispanic White, Non-Hispanic Black, Mexican American, men, women, non-drinkers, drinkers, non-smokers, ex-smokers, current smokers, BMI < 25, BMI 25–29.9, and BMI ≥ 30 (Table 2).

4. Discussion

In this sample of the US population, we documented that serum GGT concentrations within its normal range are strongly and positively associated with serum CRP levels. This positive association was consistently demonstrated in all subgroups we examined. However, serum ALT, an enzyme more specific to the liver, was not positively associated with serum CRP. It is not possible to discern causal direction of

Table 1
Association between serum gamma glutamyltransferase (GGT) and C-reactive protein (CRP) after adjustment for after adjustment for race, sex, age, cigarette smoking, alcohol consumption, and body mass index

Deciles of serum GGT (number of subjects)	Serum CRP (mg/L)		Serum CRP ≥3.0 (mg/L)		Serum CRP ≥10.0 (mg/L)	
	Geometric mean	Geometric S.D.	Odds ratio	95% CI	Odds ration	95% CI
D1 (1105)	2.85	0.06	Reference		Reference	
D2 (1452)	2.98	0.06	1.23	(1.01–1.50)	1.27	(0.89–1.83)
D3 (1130)	3.11	0.06	1.40	(1.14–1.73)	1.51	(1.05–2.19)
D4 (1398)	3.18	0.06	1.59	(1.30–1.93)	1.64	(1.16–2.33)
D5 (719)	3.22	0.08	1.62	(1.28–2.04)	1.76	(1.19–2.60)
D6 (1344)	3.20	0.06	1.61	(1.32–1.97)	1.86	(1.31–2.63)
D7 (1393)	3.45	0.06	2.17	(1.78–2.64)	2.00	(1.41–2.83)
D8 (1209)	3.52	0.07	2.38	(1.94–2.92)	2.29	(1.62–3.25)
D9 (1151)	3.62	0.07	2.45	(1.99–3.02)	2.54	(1.79–3.60)
D10 (1209)	4.12	0.08	3.41	(2.78–4.19)	3.33	(2.36–4.69)
P_{trend}	<0.01		<0.01		<0.01	

association from this cross-sectional study. However, in the CARDIA study [5], serum GGT concentrations predicted future serum CRP levels which was measured 15 years after the earlier GGT measurement and 5 years after the later in dose-response manners. They similarly predicted fibrinogen, another biomarker of inflammation and F2-isoprostanes, a highly regarded biomarker of lipid peroxidation. Taken together with other CARDIA findings of inverse associations of serum GGT with serum and dietary antioxidant vitamins and positive associations with dietary heme iron [11,12], we argue that serum GGT within its normal range is a sensitive and early marker of oxidative stress.

Supporting our argument, cellular GGT has been known to play an important role in antioxidant defense systems [13–16], although the relationship between serum GGT and cellular GGT is unknown. Cellular GGT catalyzes the initial step in the degradation of extracellular glutathione, thereby providing a supply of constituent amino acids for uptake and reutilization in intracellular glutathione synthesis. However, recent experimental studies [17–21] clearly indicate that GGT may also be involved in the generation of reactive oxygen species in the presence of iron or other transition metals.

Table 2
Geometric means of serum C-reactive protein (CRP) by quintiles of serum gamma glutamyltransferase (GGT) among subgroups after adjustment for race, sex, age, cigarette smoking, alcohol consumption, and body mass index

	Quintiles of serum GGT					P_{trend}
	Q1	Q2	Q3	Q4	Q5	
Race						
White	2.83	3.07	3.15	3.45	3.96	<0.01
Black	3.34	3.39	3.42	3.66	4.02	<0.01
Mexican	2.87	3.16	3.12	3.34	3.71	<0.01
Gender						
Men	2.82	2.81	2.84	3.01	3.45	<0.01
Women	3.16	3.44	3.54	4.09	4.42	<0.01
Alcohol						
Non-drinker	3.01	3.21	3.29	3.69	4.12	<0.01
Drinker	2.68	2.97	2.90	2.82	3.18	<0.01
Smoking						
Non-smoker	2.90	3.05	3.12	3.43	3.87	<0.01
Ex-smoker	2.93	3.16	3.32	3.64	3.93	<0.01
Current smoker	2.95	3.36	3.20	3.41	3.90	<0.01
BMI						
< 25	2.56	2.69	2.71	2.99	3.34	<0.01
25–29.9	2.83	2.96	3.04	3.30	3.74	<0.01
≥30	3.65	4.19	4.27	4.75	5.11	<0.01

Although highly elevated serum GGT has been commonly used as a marker of alcohol consumption or liver dysfunction [10], population-based studies [3–9] have observed a strong association between serum GGT levels, mostly within normal range, and many cardiovascular disease risk factors. The factors showing a positive association with elevated serum GGT level in the population studies include: old age, male gender, body mass index, smoking, lack of exercise, high blood pressure, heart rate, high blood cholesterol, high blood fasting triglycerides, high blood LDL cholesterol, low blood HDL cholesterol, high fasting glucose, and, among women, menopause and use of oral contraceptive [3–9]. In addition, baseline serum GGT level was an independent risk factor for the development of heart disease, hypertension, stroke, and type 2 diabetes [4–9]. The associations of serum GGT within normal range with many cardiovascular risk factors and/or events might be explained by a mechanism related to oxidative stress.

On the other hand, the prognostic value of serum GGT for cardiac death and non-fatal infarction was confirmed among ischemic patients with established coronary atherosclerosis and previous myocardial infarction [26]. The significance of serum GGT was more evident among patients with vulnerable plaques. The authors suggested that potent catalysis of low-density lipoprotein oxidation through the ability of GGT to enhance iron reduction by GSH might be an important mechanism in atherosclerosis [27]. They also reported the ability of GGT-mediated GSH catabolism to stimulate the reductive delocalization of iron ions bound to transferrin, a physiological source of iron [28].

Compelling evidence for the importance of inflammation and atherosclerosis at both the basic and clinical level has evolved in parallel [1,2]. To date, elevated level of several

inflammation mediators among apparently healthy men and women have proven to have predictive value for future vascular events. For clinical purposes, the most promising inflammatory biomarker appears to be CRP, a classical acute-phase marker and a member of the pentraxin family of innate immune response proteins. More than a dozen population-based studies have demonstrated that baseline CRP levels predict future cardiovascular events. CRP testing may thus have a major adjunctive role in the global assessment of cardiovascular risk.

Oxidative stress has been implicated in initiating inflammatory response through chromatin modelling (histone acetylation/deacetylation), the activation of transcription factors such as nuclear factor-kappaB and activator protein-1 leading to gene expression of pro-inflammatory mediators [21–23]. Our current and previous studies [5] suggest that elevation of serum GGT is involved in the inflammatory response. It is plausible that elevation in GGT might occur before elevation in CRP, if oxidative stress leads to an inflammatory response.

At present, both experimental and epidemiological studies on serum or cellular GGT are at a beginning stage. The strong association of serum GGT and CRP suggest that further studies on cellular or serum GGT might help to elucidate the association between oxidative stress and inflammation.

References

[1] Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135–43.

[2] Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363–9.

[3] Nilssen O, Forde OH, Brenn T. The Tromso study distribution and population determinants of gamma-glutamyltransferase. Am J Epidemiol 1990;132:318–26.

[4] Lee DH, Ha MH, Kim JH, et al. Gamma-glutamyltransferase and diabetes—A 4 year follow-up study. Diabetologia 2003;46:359–64.

[5] Lee DH, Jacobs DR, Gross M, et al. Gamma glutamyltransferase is a predictor of incident diabetes and hypertension: the CARDIA study. Clin Chem 2003;49:1358–66.

[6] Wannamethee G, Ebrahim S, Shaper AG. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. Am J Epidemiol 1995;142:699–708.

[7] Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 1998;21:732–7.

[8] Miura K, Nakagawa H, Nakamura H, et al. Serum gamma-glutamyl transferase level in predicting hypertension among male drinkers. J Hum Hypertens 1994;8:445–9.

[9] Jousilahti P, Rastenyte D, Tuomilehto J. Serum gamma-glutamyl transferase, self-reported alcohol drinking, and the risk of stroke. Stroke 2000;31:1851–5.

[10] Teschke R, Brand A, Strohmeyer G. Induction of hepatic microsomal gamma-glutamyltransferase activity following chronic alcohol consumption. Biochem Biophys Res Commun 1977;75:718–24.

[11] Lee DH, Gross M, Jacobs DR. The association of serum carotenoids and tocopherols with gamma glutamyltransferase: the CARDIA study. Clin Chem 2004;50:582–8.

[12] Lee DH, Steffen LM, Jacobs DR. Association between serum gamma-glutamyltransferase and dietary factors: CARDIA study. Am J Clin Nutr 2004;79:600–5.

[13] Shi M, Gozal E, Choy HA, Forman HJ. Extracellular glutathione and gamma-glutamyl transpeptidase prevent H_2O_2-induced injury by 2,3-dimethoxy-14-naphthoquinone. Free Radic Biol Med 1993;15:57–67.

[14] Kugelman A, Choy HA, Liu R, et al. Gamma-glutamyl transpeptidase is increased by oxidative stress in rat alveolar L2 epithelial cells. Am J Respir Cell Mol Biol 1994;11:586–92.

[15] Takahashi Y, Oakes SM, Williams MC, et al. Nitrogen dioxide exposure activates gamma-glutamyl transferase gene expression in rat lung. Toxicol Appl Pharmacol 1997;143:388–96.

[16] Karp DR, Shimooku K, Lipsky PE. Expression of gamma-glutamyl transpeptidase protects ramos B cells from oxidation-induced cell death. J Biol Chem 2001;276:3798–804.

[17] Stark AA, Russell JJ, Langenbach R, et al. Localization of oxidative damage by a glutathione-gamma-glutamyl transpeptidase system in preneoplastic lesions in sections of livers from carcinogen-treated rats. Carcinogenesis 1994;15:343–8.

[18] Zalit I, Glass GA, Stark AA. The role of chelators in the catalysis of glutathione-gamma-glutamyl transpeptidase dependent lipid peroxidation by transition metals. Biochem Mol Biol Int 1996;40:1123–33.

[19] Glass GA, Stark AA. Promotion of glutathione-gamma-glutamyl transpeptidase-dependent lipid peroxidation by copper and ceruloplasmin: the requirement for iron and the effects of antioxidants and antioxidant enzymes. Environ Mol Mutagen 1997;29:73–80.

[20] Drozdz R, Parmentier C, Hachad H, et al. Gamma-glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. Free Radic Biol Med 1998;25:786–92.

[21] Dominici S, Valentini M, Maellaro E, et al. Redox modulation of cell surface protein thiols in U937 lymphoma cells: the role of gamma-glutamyl transpeptidase-dependent H_2O_2 production and S-thiolation. Free Radic Biol Med 1999;27:623–35.

[22] Pleiner J, Mittermayer F, Schaller G, et al. Inflammation-induced vasoconstrictor hyporeactivity is caused by oxidative stress. J Am Coll Cardiol 2003;42:1656–62.

[23] MacNee W. Oxidative stress and lung inflammation in airways disease. Eur J Pharmacol 2001;429(1–3):195–207.

[24] Rahman I, MacNee W. Oxidative stress and regulation of glutathione in lung inflammation. Eur Respir J 2000;16:534–54.

[25] National Center for Health Statistics US Department of Health and Human Services. Third National Health and Nutrition Examination Survey, 1988–1994. References manuals and reports (CD-ROM). Hyattsville, MD: Centers for Disease Control and Prevention, 1996.

[26] Emdin M, Passino C, Michelassi C, et al. Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction. Eur Heart J 2001;22:1802–7.

[27] Paolicchi A, Minotti G, Tonarelli P, et al. Gamma-glutamyl transpeptidase-dependent iron reduction and low density lipoprotein oxidation-a potential mechanism in atherosclerosis. J Invest Med 1999;47:151–60.

[28] Dominici S, Paolicchi A, Lorenzini E, et al. Gamma-glutamyltransferase-dependent prooxidant reactions: a factor in multiple processes. Biofactors 2003;17:187–98.