

The Relationship Between Gamma-Glutamyltransferase (GGT), Bilirubin (Bil) and Small Dense Low-Density Lipoprotein (sdLDL) in Asymptomatic Subjects Attending a Clinic for Screening Dyslipidaemias

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Abstract

Introduction: Gamma-glutamyltransferase (GGT), bilirubin (Bil) and small dense low-density lipoprotein (sdLDL) particles are each known to be risk markers for cardiometabolic diseases which are characterised by oxidative stress conditions. These markers are connected with the oxidative milieu; however, the association between GGT, Bil, and sdLDL has been hardly examined. This hospital-based study investigated the association between GGT and sdLDL, as well as the association between Bil and sdLDL, in asymptomatic subjects. **Materials and Methods:** Cardiometabolic variables, GGT, Bil and the mean LDL particle size were measured in 100 asymptomatic subjects attending a clinic for screening dyslipidaemias (36 men and 64 women, mean age 64 years). Correlation analyses of the association between the mean LDL particle size and other variables, such as GGT and Bil, were performed. **Results:** The mean (standard deviation) levels of GGT, Bil, and the mean LDL particle size were found to be 21.7 (8.3) IU/L, 14.0 (4.3) μ mol/L, and 26.7 (0.6) nm, respectively. An univariate correlation test showed both a significant inverse correlation between the mean LDL particle size and GGT ($r = -0.33$, $P < 0.01$) and a significant positive correlation between the mean LDL particle size and Bil ($r = 0.32$, $P < 0.01$). A multiple regression analysis revealed similarly significant results of their correlations, independent of the other cardiometabolic variables. **Conclusion:** These results suggest that the correlation of GGT and sdLDL, as well as that of Bil and sdLDL, may be cooperatively associated with cardiometabolic processes. Further research is warranted in order to confirm the observed association.

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Key words: Atherosclerosis, LDL particle size, Oxidative stress, γ GT, Total bilirubin

Introduction

Oxidative stress, which is caused by an oxidant-antioxidant imbalance, is an emerging risk factor for the development of cardiovascular disease (CVD), a crucial health problem in Western countries, Asia and other developing countries.¹ Therefore, understanding oxidative stress conditions and developing a therapeutic approach to limit the effects of oxidative stress are crucial for the prevention of CVD.^{2,3} Recently, 2 serum markers, gamma-glutamyltransferase (GGT) and bilirubin (Bil), which are reflective of hepatic function in general, have been recognised as oxidative stress and antioxidative markers.⁴⁻¹⁰ In fact, GGT and Bil levels are shown to be associated with the development of cardiometabolic diseases,⁴⁻¹⁰ while the underlying mechanisms of the association between GGT and CVD,

as well as the underlying mechanisms of the association between Bil and CVD, are not yet sufficiently established.

The presence of small, dense low-density lipoprotein (sdLDL) particles, which exhibit both a small LDL particle size and a greater susceptibility to oxidation, in the blood is considered to be a risk marker of CVD.^{11,12} We have noted that subjects with insulin resistance and metabolic syndrome (oxidative stress conditions that are often concomitantly accompanied by hepatic dysfunction) display an atherogenic lipoprotein profile that includes the presence of sdLDL particles.¹³⁻¹⁵ Subjects with metabolic syndrome can have high GGT levels and low Bil levels.^{6,7,10} Circulating GGT is also thought to bind to lipoproteins, including LDL particles.⁷ In addition, a few studies indicate that the blood

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work of subjects with Gilbert's syndrome (having a total Bil level $>34.2 \mu\text{mol/L}^6$) displays fewer sdLDL particles and shows an inverse correlation between Bil and sdLDL.^{16,17}

The associations among GGT, Bil, and sdLDL may explain a possible link between these markers and CVD; however, these associations have yet to be examined. Given this background, the present study is intended to investigate the association between GGT and sdLDL, as well as the association between Bil and sdLDL, in asymptomatic subjects.

Materials and Methods

A total of 100 asymptomatic subjects attending a clinic for screening dyslipidaemias (36 men and 64 women with a mean age of 64 years) were enrolled in this hospital-based study. The inclusion criteria were as follows: subjects who were non-smokers, abstainers from alcohol, and subjects who were not taking any medications. The exclusion criteria excluded subjects who were pregnant, subjects with acute infections, subjects with a history of diabetes and subjects with a history of cardio/cerebrovascular, thyroid, collagen, severe kidney, or viral liver disease. Subjects with serum levels of alanine transaminase and aspartate transaminase $<39 \text{ IU/L}$ and GGT $<40 \text{ IU/L}$ were recruited in consideration of an approximate upper limit of their reference values, based on the pertinent literature and our experience.^{7,18} The clinical characteristics of the studied subjects are listed in Table 1. The study was approved by the institutional ethics committee and all subjects gave their informed consent.

Both the systolic blood pressure (SBP) and the diastolic

blood pressure (DBP) were measured on the subject's right arm using a sphygmomanometer while the subject was in a seated position and after the subject had rested for 5 minutes. The body mass index (BMI) was calculated as the weight divided by the square of the height while wearing light clothes. Blood was sampled after a 10-hour overnight fast. The subjects' plasma glucose levels and serum lipid panels, including LDL-cholesterol (LDL-C), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C), were measured enzymatically (reagents: Kyowa Medex Co. Ltd., Tokyo, Japan). Bil (reagent: Kainos Laboratory Inc., Tokyo, Japan) and GGT (reagent: Kyowa Medex Co. Ltd., Tokyo, Japan) were both measured with enzyme methods that were implemented in an autoanalyser (AU5800 system; Beckman Coulter Co. Ltd., Tokyo, Japan). The intra- and inter-assay coefficients of variation (CV) were found to be 1.0% and 1.0% in Bil and 1.2% and 1.1% in GGT, respectively. The mean LDL particle size was measured using a high-resolution, non-gradient polyacrylamide gel electrophoresis system (Lipoprint system; Quantimetrix, Redondo Beach, CA, USA)¹⁹ that was validated using the gold standard method of nuclear magnetic resonance spectroscopy. After the serum samples (25 μL) were photopolymerised, the samples and loading gels were briefly applied to gel tubes and then electrophoresed. The scanning system then calculated the mean LDL particle size based on the fractionalisation of the lipoproteins.¹⁹

The data are expressed as either the mean $\pm$ standard deviation or the median plus interquartile ranges. A Pearson's correlation test with a simple univariate analysis and a multiple regression analysis that was adjusted for all of the measured variables were utilised to observe the correlation between the mean LDL particle size and the other atherosclerotic risk variables, including GGT and Bil. Only the SBP was entered into the multiple regression analysis as a result of a close colineality of the SBP and the DBP. The TG values were log-transformed for all of the analyses because of their skewed distribution. A *P* value of <0.05 was considered to be statistically significant.

Results

The correlations between the mean LDL particle size and the other atherosclerotic risk variables, such as GGT and Bil, are listed in Table 2. A univariate correlation test showed that the mean LDL particle size was significantly and positively correlated with both HDL-C and Bil, while the mean LDL particle size was significantly and inversely correlated with age, BMI, SBP, TG, glucose and GGT.

A subsequent multiple regression analysis revealed both an independent, significant, and inverse correlation between the mean LDL particle size and GGT and an independent, significant, and positive correlation between the mean LDL

Table 1. Clinical Characteristics of Study Subjects

Variables	Levels*
Age, years	59 $\pm$ 11
Men/women, number	36/64
Body mass index, kg/m^2	23.1 $\pm$ 3.1
Systolic blood pressure, mmHg	135 $\pm$ 20
Diastolic blood pressure, mmHg	77 $\pm$ 10
LDL-cholesterol, mmol/L	4.28 $\pm$ 0.91
HDL-cholesterol, mmol/L	1.76 $\pm$ 0.50
Triglycerides, mmol/L	1.20 (0.84 – 1.73)
Plasma glucose, mmol/L	5.97 $\pm$ 1.57
Mean LDL particle size, nm	26.7 $\pm$ 0.6
GGT, IU/L	21.7 $\pm$ 8.3
Bilirubin, $\mu\text{mol/L}$	14.0 $\pm$ 4.3

GGT: Gamma-glutamyltransferase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein

*Values are expressed as the mean $\pm$ standard deviation, median (interquartile range) or number

Table 2. Correlation of the Mean LDL Particle Size (Outcome Variable) with Other Atherosclerotic Risk Variables, such as GGT and Bilirubin

Variables	r (P value)*	For GGT	For Bilirubin
		β (P value)*	β (P value)*
Age, years	− 0.22 (0.03)†	− 0.12 (0.19)	− 0.08 (0.34)
Gender, male	− 0.14 (0.17)	0.12 (0.19)	0.09 (0.32)
Body mass index, kg/m ²	− 0.42 (<0.01)‡	− 0.08 (0.37)	− 0.10 (0.29)
Systolic blood pressure, mmHg	− 0.28 (<0.01)‡	− 0.04 (0.65)	− 0.04 (0.67)
Diastolic blood pressure, mmHg	− 0.19 (0.07)	—	—
LDL-cholesterol, mmol/L	− 0.03 (0.77)	− 0.11 (0.17)	− 0.11 (0.19)
HDL-cholesterol, mmol/L	0.41 (<0.01)‡	0.24 (0.01)†	0.21 (0.03)†
Triglycerides, mmol/L	− 0.64 (<0.01)‡	− 0.43 (<0.01)‡	− 0.44 (<0.01)‡
Plasma glucose, mmol/L	− 0.27 (<0.01)‡	− 0.12 (0.18)	− 0.13 (0.13)
GGT, IU/L	− 0.33 (<0.01)‡	− 0.17 (0.048)†	—
Bilirubin, μ mol/L	0.32 (<0.01)‡	—	0.15 (0.049)†

GGT: Gamma-glutamyltransferase; HDL: High-density lipoprotein; LDL: Low-density lipoprotein

*Values are expressed as r (Pearson's correlation coefficient) and β (coefficient by multiple regression analysis, adjusted for age, gender, systolic blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides and glucose).

†P < 0.05

‡P < 0.01

particle size and Bil. In addition, both an independent, significant, and inverse correlation between the mean LDL particle size and TG and an independent, significant, and positive correlation between the mean LDL particle size and HDL-C were commonly seen in the multiple regression analyses that were done to examine the correlation between the LDL particle size and GGT or Bil.

Discussion

This study showed that, in asymptomatic subjects, GGT was independently, significantly and inversely correlated with the mean LDL particle size, while Bil was independently, significantly and positively correlated with the mean LDL particle size. A significant correlation between sdLDL and TG or HDL-C is an expected result, based on the prior literature.^{11,12} The findings of a significant association between GGT, Bil and the mean LDL particle size suggest that GGT and sdLDL, as well as Bil and sdLDL, may be cooperatively involved in atherogenesis. While the clinical relevance of these findings should be further confirmed with future studies, our present findings of significant relationships of GGT and Bil with sdLDL may provide new insights into the association between GGT and CVD, as well as the association between Bil and CVD. As a result, these findings are meaningful as both GGT and Bil are recently attractive markers of CVD that are measurable in daily practice, as well as the underlying mechanisms of the association between GGT and Bil on incident CVD remain to be explored.⁴⁻¹⁰ In addition, the result of an inverse correlation between Bil and sdLDL appears to be similar to findings in subjects with Gilbert's

syndrome (who differ from our study population).^{16,17} Thus, of note, our present study will extend the knowledge currently available regarding Bil and sdLDL.

Although we do not fully know whether GGT is an innocent bystander or a causal contributor to the development of CVD,⁷ we do know that GGT participates in glutathione metabolism and in the extracellular degradation of the antioxidant glutathione GSH, thereby triggering a pro-oxidant action.⁷ Bil is the major product of heme metabolism and plays a role in cellular protection by scavenging for peroxyl radicals (this antioxidant capacity is attributable to any of the Bil forms).⁴ GGT (having an oxidative property) and Bil (having an antioxidant property) are both inversely associated with oxidative stress conditions.⁷ Oxidative stress can potentially induce regional and/or systemic insulin resistance,²⁰ while insulin resistance can also induce oxidative stress.²¹ Oxidative stress and insulin resistance are associated with the overproduction of VLDL in the liver and the bloodstream, thereby producing sdLDL (in part by modulating the activities of lipoprotein lipase and hepatic lipase).¹¹⁻¹⁵ Existing sdLDL can be involved in vascular atherogenesis, which causes further oxidative stress in the atherosclerotic lesions.^{11,12} Subsequently, GGT and Bil may also respond to the oxidative stress states. Thus, these interrelationships highlight a vicious cycle of the oxidative milieu that may partly explain the results of our present study.

The limitations of this study included a small sample size and a cross-sectional design. Our study setting (i.e. hospital-based screening for specific conditions such as dyslipidaemias) might restrict generalisation of the results.

In addition, data on CVD-related outcomes were not available. These issues must be addressed in future studies in order to generalise the results of this study.

Conclusion

In conclusion, among asymptomatic subjects attending a clinic for screening dyslipidaemias, the mean LDL particle size was both independently, significantly, and inversely correlated with GGT and independently, significantly, and positively correlated with Bil. This suggests that GGT and sdLDL, as well as Bil, may be cooperatively involved in atherogenesis. Further research is needed to confirm these findings.

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