Original Article

# Elevated Serum Gamma-glutamyl Transpeptidase Levels and Fatty Liver Strongly Predict the Presence of Carotid Plaque

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***Aim*: There is a strong relationship between carotid atherosclerosis and future cardiovascular disease (CVD). This study sought to clarify the association of fatty liver and an elevated serum gamma-glutamyl transpeptidase (GGT) level with carotid atherosclerosis.**
***Methods*: We reviewed the medical records of subjects who underwent medical checkups at our institute. Carotid atherosclerosis and fatty liver were assessed using ultrasound (US), and predictors of increased carotid intima-media thickness (IMT) and carotid plaque were identified using a logistic regression model.**
***Results*: In total, 958 subjects (564 men, 394 women; median age, 59 years) were enrolled. The median value of the mean carotid IMT was 0.713 mm, and the frequency of carotid plaque was 19.5%. For the highest quartile of the mean carotid IMT (≥0.863 mm), a male sex, older age, hypertension (HT), dyslipidemia (DL) and type 2 diabetes mellitus (DM) were identified as independent predictors. A male sex, older age, HT and elevated serum GGT level were found to be significant predictors of the presence of carotid plaque. In addition, fatty liver correlated with the existence of carotid plaque. When the combination of the serum GGT level and presence or absence of fatty liver was included as a variable in the analysis, a male sex, older age, HT and fatty liver with a serum GGT level of ≥83 IU/L (90th percentile) (odds ratio 3.21, 95% confidence interval 1.27–8.12, $p$=0.014) were identified to be significantly associated with carotid plaque.**
***Conclusions*: This study suggests that the simultaneous presence of an elevated serum GGT level and fatty liver is highly predictive of carotid plaque.**

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**Key words:** Fatty liver, Gamma-glutamyl transpeptidase, Carotid plaque

## Introduction

Atherosclerosis underlies the major pathogenesis of cardiovascular disease (CVD), which is the leading cause of death worldwide and responsible for approximately 30% of all deaths[1, 2]. Therefore, assessing atherosclerosis is essential for predicting and preventing CVD in apparently healthy subjects. Due to its accessibility, simplicity and reproducibility, carotid ultrasound (US) is the most widely used tool for evaluating the degree of generalized atherosclerosis[3], and there is a strong relationship between the detection of carotid atherosclerosis and future CVD events[4-6].

Previous studies have demonstrated that multiple factors, such as aging, hypertension (HT), dyslipidemia (DL), type 2 diabetes mellitus (DM) and smoking, are associated with carotid atherosclerosis[7]. Recently, other associated factors have been identified. The number of individuals with fatty liver has been increasing worldwide, and fatty liver has become the most common

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liver disorder, with an estimated prevalence of 20% to 30% in the general population[8]. Furthermore, epidemiological studies have revealed a strong association between fatty liver and carotid atherosclerosis[9, 10], and accumulating data indicate that elevated serum gamma-glutamyl transpeptidase (GGT) levels are correlated with carotid atherosclerosis[11, 12] and that GGT per se may play a direct role in the development of atherosclerosis[13]. In this context, studies have been conducted to determine whether an elevated serum GGT level and fatty liver are predictive of future CVD events. Some studies have examined the impact of elevated serum GGT levels on all-cause and CVD mortality and showed positive results[14, 15], and similar results have been reported regarding the relationship between fatty liver and CVD events[16]. Notably, one study found that the serum GGT levels are significantly associated with all-cause and CVD mortality in men and that this association is stronger among those with increased echogenicity of the liver parenchyma, known as the US sign of fatty liver[17].

## Aim

In the current study, we hypothesized that the simultaneous presence of fatty liver and an elevated serum GGT level may therefore be more strongly associated with atherosclerosis than the presence of either variable alone. Hence, we determined predictors of carotid atherosclerosis using a logistic regression model that included these two parameters.

## Methods

### Subjects

We reviewed the medical records of subjects who consecutively underwent medical checkups at our health evaluation center between 2011 and 2013. For subjects who underwent repeated medical checkups, we used their most recent records. The exclusion criteria included cases of insufficient recorded data, hepatitis B or C virus infection, a past and/or current history of cardiovascular or liver disease and past and/or current medications capable of influencing the course of atherosclerosis, such as anti-diabetic agents, antihypertensive agents, DL agents and antiplatelet/anticoagulant agents. The study protocol was approved by the Ethics Committee of Kanazawa Medical University (approval no. 144) and conducted in accordance with the Declaration of Helsinki.

### Laboratory Tests

The serum total cholesterol levels were measured using the cholesterol oxidase method (Kyowa Medex Co., Ltd, Tokyo, Japan). The serum low-density-lipoprotein (LDL) and high-density-lipoprotein (HDL) cholesterol levels were measured according to the direct method (Sekisui Medical Co., Ltd, Tokyo, Japan), and the serum triglyceride levels were measured using an enzymatic color test (Kyowa Medex Co., Ltd). Additionally, the fasting blood glucose levels were assessed using the hexokinase method (Shino-Test Corporation, Tokyo, Japan), the hemoglobin A1c (HbA1c) levels were determined via high-performance liquid chromatography (HPLC) (Tosoh Corporation, Tokyo, Japan) and the serum alanine aminotransferase and GGT levels were measured using the Japan Society of Clinical Chemistry (JSCC) standardization method (Kanto Chemical Co., Inc., Tokyo, Japan).

### Definitions of HT, DL, DM and Metabolic Syndrome

HT was defined according to the Japanese Society of Hypertension guidelines for the management of HT, as follows: a systolic blood pressure of ≥140 mmHg or diastolic blood pressure of ≥90 mmHg[18]. DL was defined as a serum LDL cholesterol level of ≥140 mg/dL, serum HDL cholesterol level of <40 mg/dL or serum triglyceride level of ≥150 mg/dL, according to the criteria of the Japan Atherosclerosis Society[19]. DM was defined according to the guidelines of the Japan Diabetes Society, as follows: a fasting blood glucose level of ≥126 mg/dL and HbA1c level of ≥6.5%[20]. Metabolic syndrome was defined according to the Japanese criteria, as follows: a waist circumference of ≥85 cm for men and ≥90 cm for women and two or more of the following items, a serum triglyceride level of ≥150 mg/dL and/or serum HDL cholesterol level of <40 mg/dL, systolic blood pressure of ≥130 mmHg and/or diastolic blood pressure of ≥85 mmHg or a fasting blood glucose level of ≥110 mg/dL[21].

### Assessment of Carotid Atherosclerosis and Fatty Liver

Experienced ultrasonographers who were blinded to the medical checkup data of each subject performed the carotid and abdominal US examinations. High-resolution US machines (Aplio XG/500/80, Toshiba Medical Systems Corporation, Tochigi, Japan) and a 5-MHz probe were used for abdominal US; the same US machines and an 8.4- or 9-MHz probe were used for carotid US. The mean carotid intima-media thickness (IMT) was calculated by averaging six or eight measurements obtained from both sides of the far walls of the common carotid arteries. Carotid plaque

**Table 1.** Study subject characteristics ($n=958$)

| Variable | |
|---|---|
| Sex, male/female | 564/394 |
| Age, years | 59 (44, 71) |
| BMI, kg/m$^2$ | 22.8 (19.2, 26.9) |
| BMI ≥ 25 kg/m$^2$, n (%) | 212 (22.1) |
| Systolic blood pressure, mmHg | 123 (102, 145) |
| Diastolic blood pressure, mmHg | 74 (60, 89) |
| Hypertension, n (%) | 171 (17.8) |
| TC, mg/dL | 213 (171, 257) |
| LDL, mg/dL | 122 (84, 159) |
| HDL, mg/dL | 56 (40, 79) |
| TG, mg/dL | 99 (55, 209) |
| Dyslipidemia, n (%) | 443 (46.2) |
| FBG, mg/dL | 95 (85, 111) |
| HbA1c, % | 5.6 (5.2, 6.1) |
| Type 2 diabetes mellitus, n (%) | 19 (2.0) |
| Metabolic syndrome, n (%) | 110 (11.5) |
| ALT, IU/L | 20 (13, 41) |
| GGT, IU/L | 27 (14, 83) |
| Fatty liver, n (%) | 206 (21.5) |
| Current smoking, n (%) | 187 (19.5) |
| Alcohol consumption, ≤ 20/ > 20, < 60/ ≥ 60, g/day | 602/307/49 |
| Carotid IMT, mm | 0.713 (0.563, 0.963) |
| Carotid plaque, n (%) | 187 (19.5) |

Variables are expressed as the median (10, 90 percentiles).
BMI, body mass index; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase; IMT, intima-media thickness.

was defined as an IMT > 1.5 mm in any portion of the carotid arteries[22]. The diagnosis of fatty liver was made based on the detection of increased echogenicity of the liver parenchyma compared to that of the right renal cortex[23]. All US results were double checked by experienced ultrasonographers.

**Statistical Analysis**

The variables are expressed as the median (10, 90 percentile). The chi-square test or Fisher's exact probability test were used to compare categorical variables, and Student's *t*-test, the Mann–Whitney *U* test and the Kruskal-Wallis test were used to compare continuous variables. We further examined predictors of carotid atherosclerosis using a multiple logistic regression model; the objective variables included an increased mean carotid IMT (highest quartile) and the presence of carotid plaque, and the explanatory variables included sex (female/male), age (years), body mass index (BMI) (kg/m$^2$), HT (absence/presence), DL (absence/presence), DM (absence/presence), serum alanine aminotransferase level (IU/L), serum GGT level (IU/L), fatty liver (absence/presence), smoking status (nonsmoking and past smoking/current smoking) and alcohol consumption (≤ 20 g/day/ > 20, < 60 g/day/ ≥ 60 g/day). Following a univariate analysis of each variable, we performed a multivariate analysis using all variables. A $p$ value of $< 0.05$ was considered to be statistically significant. All statistical analyses were performed using the STATA version 11.1 software program (STATA Corp, College Station, TX, USA).

## Results

During the study period, 1,599 subjects underwent medical checkups at least once (813 subjects, once; 786 subjects, twice or more). Of these subjects, 56 were excluded due to insufficient data and 567 were excluded due to a past and/or current history of cardiovascular or liver disease and a past and/or current history of medications capable of influencing the

**Table 2.** Factors correlated with the highest quartile of the mean carotid IMT (≥0.863 mm) ($n$=958)

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | $p$ value | OR | 95% CI | $p$ value |
| Male (vs. female) | 2.89 | 2.07−4.02 | <0.0001 | 2.61 | 1.71−3.97 | <0.0001 |
| Age, years | 1.12 | 1.10−1.15 | <0.0001 | 1.13 | 1.11−1.16 | <0.0001 |
| BMI, kg/m$^2$ | 1.11 | 1.06−1.16 | <0.0001 | 1.07 | 0.99−1.15 | 0.054 |
| Hypertension | 2.40 | 1.69−3.40 | <0.0001 | 2.05 | 1.36−3.09 | 0.001 |
| Dyslipidemia | 1.75 | 1.30−2.35 | <0.0001 | 1.62 | 1.13−2.34 | 0.009 |
| Type 2 diabetes mellitus | 8.73 | 3.11−24.51 | <0.0001 | 5.37 | 1.70−16.92 | 0.004 |
| ALT, IU/L | 1.01 | 0.99−1.02 | 0.247 | 0.99 | 0.98−1.01 | 0.548 |
| GGT, IU/L | 1.002 | 0.99−1.005 | 0.110 | 1.001 | 0.99−1.01 | 0.542 |
| Fatty liver | 1.64 | 1.17−2.29 | 0.004 | 1.42 | 0.89−2.26 | 0.133 |
| Current smoking (vs. nonsmoking and past smoking) | 0.86 | 0.59−1.25 | 0.427 | 0.84 | 0.53−1.32 | 0.445 |
| Alcohol consumption, >20, <60 g/day (vs. ≤20 g/day) | 1.27 | 0.93−1.72 | 0.119 | 0.87 | 0.58−1.31 | 0.506 |
| Alcohol consumption, ≥60 g/day (vs. ≤20 g/day) | 1.07 | 0.56−2.06 | 0.642 | 0.65 | 0.28−1.51 | 0.318 |

IMT, intima-media thickness; OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

course of atherosclerosis. Of the remaining 976 subjects, eight were excluded due to hepatitis B virus infection and 10 were excluded due to hepatitis C virus infection. Therefore, 958 subjects (men 564, women 394; median age 59 years) were enrolled in this study. **Table 1** lists the subject characteristics. The median value of the mean carotid IMT was 0.713 mm, and 19.5% (187/958) of the subjects had plaque. In total, 21.5% (206/958) of the subjects had fatty liver.

In order to determine the predictors of an increased mean carotid IMT, we used the highest quartile of the mean carotid IMT (≥0.863 mm) as the objective variable. In the univariate analysis, a male sex, older age, increased BMI, HT, DL, DM and fatty liver were identified as significant variables. In the multivariate analysis, a male sex, older age, HT, DL and DM were found to be independently associated with an increased mean carotid IMT (**Table 2**). Neither fatty liver ($p$=0.133) nor an elevated serum GGT level ($p$=0.542) were significant predictors. When the analysis was performed for each sex, there were differences with respect to the predictors of an increased mean carotid IMT. In men, an older age [odds ratio (OR) 1.12, 95% confidence interval (CI) 1.09−1.16, $p$<0.0001], HT (OR 1.99, 95% CI 1.21−3.27, $p$=0.007), DL (OR 1.72, 95% CI 1.07−2.75, $p$=0.025) and DM (OR 6.09, 95% CI 1.93−19.26, $p$=0.002) were significant predictors. Furthermore, fatty liver (OR 2.26, 95% CI 1.31−3.91, $p$=0.003) was significantly associated with an increased mean carotid IMT. In contrast, in women, an older age (OR 1.16, 95% CI 1.11−1.21, $p$<0.0001) was the only significant predictor.

In the analysis of predictors of carotid plaque, a male sex, older age, HT, DL, elevated serum GGT level and increased alcohol consumption (>20, <60 g/day) were found to be significant in the univariate analysis. In the multivariate analysis, a male sex, older age, HT and elevated serum GGT level were identified as independent predictors of the presence of carotid plaque (**Table 3**). Fatty liver ($p$=0.093) and DL ($p$=0.059) trended with the presence of carotid plaque. When the analysis was performed for each sex, there were differences with respect to the predictors of carotid plaque. For example, in men, an older age (OR 1.09, 95% CI 1.06−1.11, $p$<0.0001), HT (OR 1.95, 95% CI 1.21−3.14, $p$=0.006) and elevated serum GGT level (OR 1.005, 95% CI 1.001−1.009, $p$=0.025) were significant predictors, whereas in women, an older age (OR 1.12, 95% CI 1.07−1.17, $p$<0.0001) was the only significant predictor.

To obtain the cut-off value of the serum GGT level for predicting carotid plaque, we calculated the odds ratios in the 50th, 75th and 90th percentiles. Consequently, the 90th percentile (83 IU/L) was chosen as the cut-off threshold (≥83 IU/L, OR 1.83, 95% CI 1.03−3.24, $p$=0.038). When the enrolled subjects were divided into two groups according to the cut-off value of the serum GGT level, the rates of a male sex (89.7% vs. 55.4%), DL (63.9% vs. 44.3%), fatty liver (34.0% vs. 20.1%), current smokers (33.0% vs. 18.0%), alcohol consumption (>20 g/day, 78.4% vs. 32.5%) and carotid plaque (32.0% vs. 18.1%)

**Table 3.** Factors associated with the presence of carotid plaque ($n=958$)

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | $p$ value | OR | 95% CI | $p$ value |
| Male (vs. female) | 2.70 | 1.87−3.90 | <0.0001 | 1.99 | 1.28−3.08 | 0.002 |
| Age, years | 1.08 | 1.06−1.11 | <0.0001 | 1.09 | 1.07−1.12 | <0.0001 |
| BMI, kg/m$^2$ | 1.04 | 0.99−1.09 | 0.101 | 0.98 | 0.91−1.05 | 0.606 |
| Hypertension | 2.03 | 1.40−2.96 | <0.0001 | 1.71 | 1.13−2.59 | 0.011 |
| Dyslipidemia | 1.51 | 1.10−2.09 | 0.011 | 1.43 | 0.99−2.09 | 0.059 |
| Type 2 diabetes mellitus | 2.46 | 0.95−6.34 | 0.062 | 1.32 | 0.48−3.64 | 0.588 |
| ALT, IU/L | 1.00 | 0.99−1.01 | 0.347 | 0.99 | 0.98−1.01 | 0.506 |
| GGT, IU/L | 1.006 | 1.002−1.009 | <0.0001 | 1.005 | 1.001−1.009 | 0.014 |
| Fatty liver | 1.44 | 0.99−2.08 | 0.053 | 1.50 | 0.93−2.42 | 0.093 |
| Current smoking (vs. nonsmoking and past smoking) | 1.25 | 0.85−1.84 | 0.259 | 1.19 | 0.76−1.86 | 0.460 |
| Alcohol consumption, >20, <60 g/day (vs. ≤20 g/day) | 1.60 | 1.15−2.23 | 0.005 | 1.17 | 0.78−1.75 | 0.442 |
| Alcohol consumption, ≥60 g/day (vs. ≤20 g/day) | 1.53 | 0.79−2.94 | 0.207 | 0.96 | 0.43−2.16 | 0.927 |

OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

were significantly higher among the subjects with a serum GGT level of ≥83 IU/L ($n=97$) compared to those observed in the subjects with a serum GGT level of <83 IU/L ($n=861$). Furthermore, the BMI (median 23.7 kg/m$^2$ vs. 22.7 kg/m$^2$) and serum alanine aminotransferase level (median 31 IU/L vs. 19 IU/L) were also higher in the former group than in the latter group.

The subjects were divided into four groups based on the cut-off value of the serum GGT and the presence or absence of fatty liver (**Table 4**). The ratios of male to female subjects were higher in the groups with a serum GGT level of ≥83 IU/L, fatty liver and both compared to that seen in the group without these two factors. BMI and the rates of HT and DL were also higher in the subjects with fatty liver than in the subjects without fatty liver. The number of current smokers was highest in the group without fatty liver and a serum GGT level of ≥83 IU/L, followed by the groups with fatty liver and a serum GGT level of ≥83 IU/L, fatty liver and a serum GGT level of <83 IU/L and non-fatty liver and a serum GGT level of <83 IU/L. The number of subjects with moderate to severe alcohol consumption was highest in the group with non-fatty liver and a serum GGT level of ≥83 IU/L, followed by the groups with fatty liver and a serum GGT level of ≥83 IU/L, non-fatty liver and a serum GGT level of <83 IU/L and fatty liver and a serum GGT level of <83 IU/L. The mean carotid IMT was higher in the subjects with fatty liver than in those without fatty liver, and the ratio of patients with carotid plaque was highest in the group with fatty liver and a serum GGT level of ≥83 IU/L, followed by the groups with non-fatty liver and a serum GGT level of ≥83 IU/L, fatty liver and a serum GGT level of <83 IU/L and non-fatty liver and a serum GGT level of <83 IU/L.

**Table 5** shows the predictors of carotid plaque when the combination of the serum GGT level and the presence or absence of fatty liver was included as an explanatory variable. In the univariate analysis, a male sex, older age, HT, DL, the combination of fatty liver and serum GGT ≥83 IU/L and increased alcohol consumption (>20, <60 g/day) were identified as significant variables. In the multivariate analysis, a male sex, older age, HT, fatty liver and serum GGT level of ≥83 IU/L (OR 3.21, 95% CI 1.27−8.12, $p=0.014$) were independent predictors of carotid plaque.

## Discussion

The current findings suggest that an elevated serum GGT level and fatty liver are closely associated with carotid atherosclerosis, although this association was stronger for carotid plaque than for an increased mean carotid IMT. Moreover, when the serum GGT levels and fatty liver were combined in the analysis of predictors of carotid plaque, the simultaneous presence of an elevated serum GGT level and fatty liver was significant. In contrast, an elevated serum GGT level without fatty liver and fatty liver without an elevated serum GGT level were not significant.

In the current study, we used fatty liver and the serum GGT level as two distinct variables. Although fatty liver and the serum GGT level are positively correlated with each other[24], the relationship between these two parameters is not necessarily constant. Alco-

**Table 4.** Comparison of the characteristics of the subjects stratified according to the fatty liver status and serum GGT level ($n=958$)

| Variable | Non-fatty liver and GGT <83 IU/L ($n=688$) | Fatty liver and GGT <83 IU/L ($n=173$) | Non-fatty liver and GGT ≥83 IU/L ($n=64$) |
|---|---|---|---|
| Sex, male/female | 348/340 | 129/44 | 57/7 |
| Age, years | 59 (44, 71) | 59 (44, 67) | 57 (46, 69) |
| BMI, kg/m$^2$ | 22.1 (18.6, 25.4) | 25.2 (22.4, 29.7) | 23.0 (19.8, 26.1) |
| Hypertension, n (%) | 113 (16.4) | 35 (20.2) | 11 (17.2) |
| Dyslipidemia, n (%) | 259 (37.6) | 122 (70.5) | 38 (59.4) |
| Type 2 diabetes, n (%) | 10 (1.5) | 7 (4.0) | 1 (1.6) |
| ALT, IU/L | 18 (12, 29) | 28 (16, 59) | 27.5 (18, 49) |
| GGT, IU/L | 22 (13, 52) | 32 (18, 67) | 104 (86, 221) |
| Current smoking n (%) | 114 (16.6) | 41 (23.7) | 23 (35.9) |
| Alcohol consumption, ≤20/>20, <60/≥60, g/day | 455/211/22 | 126/45/2 | 11/33/20 |
| Carotid IMT, mm | 0.700 (0.563, 0.963) | 0.738 (0.575, 1.000) | 0.688 (0.517, 0.988) |
| Carotid plaque, n (%) | 119 (17.3) | 37 (21.4) | 18 (28.1) |

| Variable | Fatty liver and GGT ≥83 IU/L ($n=33$) | $p$ value* |
|---|---|---|
| Sex, male/female | 30/3 | <0.0001 |
| Age, years | 59 (41, 66) | 0.051 |
| BMI, kg/m$^2$ | 26.0 (23.7, 30.4) | <0.0001 |
| Hypertension, n (%) | 12 (36.4) | 0.046 |
| Dyslipidemia, n (%) | 24 (72.7) | <0.0001 |
| Type 2 diabetes, n (%) | 1 (3.0) | 0.170 |
| ALT, IU/L | 42 (23, 124) | <0.0001 |
| GGT, IU/L | 108 (83, 203) | <0.0001 |
| Current smoking n (%) | 9 (27.3) | 0.0001 |
| Alcohol consumption, ≤20/>20, <60/≥60, g/day | 10/18/5 | <0.0001 |
| Carotid IMT, mm | 0.740 (0.613, 1.060) | 0.047 |
| Carotid plaque, n (%) | 13 (39.4) | 0.003 |

All variables are expressed as the median (10, 90 percentiles).
*The chi-square test or Fisher's exact probability test were used to compare categorical variables. The Kruskal-Wallis test was used to compare continuous variables.
GGT, gamma-glutamyl transpeptidase; BMI, body mass index; ALT, alanine aminotransferase; IMT, intima-media thickness.

hol consumption[25], smoking[26], an older age[27] and obesity[28] can increase the serum GGT levels and thereby modify the relationship between fatty liver and the serum GGT level. Therefore, our analyses were conducted to clarify the manner in which fatty liver and an elevated serum GGT level are associated with carotid atherosclerosis in the same logistic regression model.

We compared our results with those of previous studies investigating the association of fatty liver and an elevated serum GGT level with carotid atherosclerosis. In a systematic review analyzing the association between non-alcoholic fatty liver disease and carotid atherosclerosis, liver disease was positively correlated with both an increased carotid IMT and carotid plaque[9]. In contrast, a recent study of a Japanese population demonstrated a positive correlation between the serum GGT levels and increased carotid IMT values (although the analyses were univariate)[12]. Another large-scale study by Kozakova *et al.*, in which the subjects were free of CVD, HT, DL, DM and metabolic syndrome, examined the association between the fatty liver index (FLI) (an index used to predict the presence of fatty liver) and carotid atherosclerosis. These authors reported the following conclusions: 1) FLI is a significant predictor of an increased mean carotid IMT, but not an elevated serum GGT level, when FLI is replaced by parameters used in its equation (BMI, waist circumference, serum triglyceride level and serum GGT level) and 2) FLI is a significant predictor of carotid plaque, with an elevated serum GGT level also being identified as a significant predictor when

**Table 5.** Association of fatty liver and the serum GGT level with carotid plaque ($n=958$)

| Variable | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | OR | 95% CI | *p* value | OR | 95% CI | *p* value |
| Male (vs. female) | 2.70 | 1.87−3.90 | <0.0001 | 2.07 | 1.34−3.21 | 0.001 |
| Age, years | 1.08 | 1.06−1.11 | <0.0001 | 1.09 | 1.07−1.12 | <0.0001 |
| BMI, kg/m$^2$ | 1.04 | 0.99−1.09 | 0.101 | 0.98 | 0.91−1.05 | 0.575 |
| Hypertension | 2.03 | 1.40−2.96 | <0.0001 | 1.73 | 1.14−2.63 | 0.010 |
| Dyslipidemia | 1.51 | 1.10−2.09 | 0.011 | 1.40 | 0.97−2.04 | 0.077 |
| Type 2 diabetes mellitus | 2.46 | 0.95−6.34 | 0.062 | 1.32 | 0.47−3.68 | 0.595 |
| ALT, IU/L | 1.00 | 0.99−1.01 | 0.347 | 0.99 | 0.98−1.01 | 0.576 |
| Fatty liver and GGT <83 IU/L (vs. Non-fatty liver and GGT <83 IU/L) | 1.15 | 0.77−1.73 | 0.494 | 1.48 | 0.89−2.45 | 0.133 |
| Non-fatty liver and GGT ≥83 IU/L (vs. Non-fatty liver and GGT <83 IU/L) | 1.68 | 0.95−2.97 | 0.075 | 1.67 | 0.84−3.32 | 0.141 |
| Fatty liver and GGT ≥83 IU/L (vs. Non-fatty liver and GGT <83 IU/L) | 2.81 | 1.37−5.75 | 0.005 | 3.21 | 1.27−8.12 | 0.014 |
| Current smoking (vs. Nonsmoking and Past smoking) | 1.25 | 0.85−1.84 | 0.259 | 1.22 | 0.78−1.91 | 0.385 |
| Alcohol consumption, >20, <60 g/day (vs. ≤20 g/day) | 1.60 | 1.15−2.23 | 0.005 | 1.21 | 0.82−1.81 | 0.340 |
| Alcohol consumption, ≥60 g/day (vs. ≤20 g/day) | 1.53 | 0.79−2.94 | 0.207 | 1.09 | 0.49−2.40 | 0.837 |

IMT, intima-media thickness; OR, odds ratio; CI, confidence interval; BMI, body mass index; ALT, alanine aminotransferase; GGT, gamma-glutamyl transpeptidase.

FLI is replaced by other parameters[11]. The results of our study and the study by Kozakova *et al.* are similar in terms of the close association between an elevated serum GGT level and carotid plaque. Our results also suggest that the presence of fatty liver reinforces this association. Conversely, an elevated serum GGT level enhances the association between fatty liver and carotid plaque. In our cohort, traditional factors, including an older age, HT, DL and DM, were more strongly associated with an increased mean carotid IMT than fatty liver.

In the current study, the results revealed sex differences with respect to the predictors of carotid atherosclerosis. For example, in men, fatty liver and an elevated serum GGT level were identified to be predictors of an increased mean carotid IMT and carotid plaque, whereas in women, neither of these two parameters were significantly associated with carotid atherosclerosis. These findings are in agreement with those of previous studies in which the association of an elevated serum GGT level with oxidative stress[12] and that of an elevated serum GGT level and fatty liver with CVD mortality[17] were found to be stronger in men than in women. Future studies are needed to clarify the reasons underlying these sex differences.

Accumulating data suggest that GGT per se may play a role in the progression of atherosclerosis. Previous studies have demonstrated the presence of GGT-positive foam cells in atherosclerotic plaques[29, 30]. In addition, another study by Franzini *et al.* examined human carotid plaques and revealed the presence of glutathione, cysteinyl-glycine, cysteine and LDL/GGT complexes[31]. Cysteinyl-glycine, a dipeptide derived from GGT-mediated glutathione degradation, promotes LDL oxidation via an iron reduction reaction that results in the production of superoxide radicals. This GGT-induced LDL oxidation may be a possible underlying mechanism of atherosclerotic progression[13]. The study by Franzini *et al.* also demonstrated a positive correlation between the serum GGT level and GGT activity in atherosclerotic plaques[31]. Our results showing an elevated serum GGT level to be associated with carotid plaque are in agreement with the above findings, and this association supports the concept of the presumptive pathological mechanism of atherosclerosis.

The role of fatty liver in atherosclerosis, particularly the causal relationship between fatty liver and atherosclerosis, remains to be fully elucidated. Some researchers believe fatty liver is a cause of atherosclerosis, whereas some researchers believe that fatty liver is only a bystander to atherosclerosis. Various evidence supports the first hypothesis, as the putative underlying mechanisms of atherosclerosis in non-alcoholic fatty liver disease include insulin resistance, atherogenic DL, chronic inflammation (as represented by elevated serum levels of C-reactive protein, interleukin-6 and tumor necrosis factor $\alpha$), hypercoagulation and hypofibrinolysis (as represented by elevated serum levels of fibrinogen, factor VII and plasminogen activator inhibitor 1)[32]. In particular, one study examined the associations between non-alcoholic steatohepatitis

and conditions related to atherosclerosis in overweight men with non-alcoholic steatohepatitis and those without steatosis but with similar levels of visceral adiposity. That study demonstrated higher serum levels of high-sensitivity C-reactive protein, fibrinogen and plasminogen activator inhibitor 1 and lower levels of adiponectin (a parameter of insulin sensitivity) in the former group than in the latter group, which suggests that non-alcoholic steatohepatitis may be directly associated with atherosclerosis[33]. Given these previous findings and our current results, an elevated serum GGT level and fatty liver may synergistically contribute to the development of atherosclerotic plaque.

This study is associated with some limitations. First, because this was a cross-sectional study, we cannot prove the causal links between carotid atherosclerosis and its predictors. Second, fatty liver was diagnosed based on US findings. Studies have shown that the diagnostic accuracy of the US method is excellent for moderate to severe fatty liver (sensitivity, 82% to 100%; specificity, 98%), although it is somewhat lower for mild fatty liver (sensitivity, 53% to 67%; specificity, 77% to 93%)[23]. Therefore, the application of US might have affected our results. Third, we did not determine the degree of steatosis or the presence or absence of steatohepatitis. Previous studies have shown a positive relationship between the stage of non-alcoholic fatty liver disease and the carotid IMT[34]. A recent study demonstrated a close association between an elevated serum GGT level and the stage of non-alcoholic steatohepatitis in DM subjects[35]. Future analyses are required to clarify whether the combination of fatty liver diagnosed on US and the serum GGT level can be used as a surrogate marker of the severity and stage of fatty liver disease. Fourth, the present study population included only Japanese subjects, thereby limiting the extrapolation of our results to other ethnic groups.

## Conclusion

This study suggests that the simultaneous presence of an elevated serum GGT level and fatty liver is highly predictive of carotid plaque and can serve as an effective marker of the risk of future CVD events. Therefore, routine carotid US should be performed in subjects with these conditions. Future clinical and experimental studies will help to reveal the causal relationship between an elevated serum GGT level, fatty liver and atherosclerosis and clarify the underlying pathological mechanisms.

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## Conflicts of Interest

The authors have no conflicts of interest to declare regarding the current manuscript.

## Author Contributions

Dr. Toshikuni, Dr. Nagasawa, Dr. Uenishi, Dr. Asaji and Dr. Tsutsumi contributed to the study design. Dr. Nakanishi, Dr. Uenishi, Dr. Nagasawa and Dr. Asaji performed the data collection. Dr. Toshikuni performed the data analysis. Dr. Toshikuni wrote the manuscript. Dr. Tsutsumi supervised the work. All authors approved the final version of the manuscript.

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