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ORIGINAL ARTICLE

경동맥 죽상동맥경화증과 Alanine Aminotransferase의 독립적 연관성

전북대학교병원 신경과*, 전북대학교 의학전문대학원 신경과학교실†

남도현* 양태호* 신현준* 소기윤* 오선영*† 신병수*† 서만욱*† 정슬기*†

Alanine Aminotransferase is Independently Associated with Carotid Atherosclerosis

Do-Hyun Nam, MD*, Tae-Ho Yang, MD*, Hyun-June Shin, MD*, Ki Youn So, MD*, Sun-Young Oh, MD, PhD*†, Byeong Soo Shin, MD, PhD*†, Man-Wook Seo, MD, PhD*†, Seul-Ki Jeong, MD, PhD*†

Department of Neurology, Biomedical Institute of Chonbuk National University Hospital, †Department of Neurology and Research Institute of Clinical Medicine, Chonbuk National University-Biomedical Institute of Chonbuk National University Hospital, Jeonju, Korea

ABSTRACT

Background: Alanine aminotrasferase (ALT) has been reported to be independently associated with cardiovascular diseases and vascular mortality. The aim of the present study was to explore whether ALT could be associated with carotid atherosclerosis, if factors which could affect liver function were considered. **Methods**: The present cross-sectional study included 293 subjects who underwent abdominal and carotid ultrasonographic examinations and biochemical investigations. Among them, we excluded participants who had any of the exclusion criteria of nonalcoholic fatty liver disease (NAFLD). Regression analyses were performed for ALT and carotid intima-media thickness (IMT) and plaque, and receiver operating characteristic (ROC) curves were analyzed. **Results**: In total, 216 (73.7%) met all the criteria for the NAFLD-eligibility and were included. ALT was independently associated with carotid IMT and plaque, even after adjusting for age, sex, and all the possible potential confounders. A unit (IU/L) increase of ALT for carotid plaque showed an odd ratio of 1.05 and 95% confidence intervals; 1.01-1.09. The area under ROC curves for carotid plaque of ALT was 0.69, which was significantly higher than that of NAFLD (0.58, p=0.032). **Conclusions**: ALT was significantly and independently associated with both carotid IMT and plaque in the general population who are eligible for a diagnosis of NAFLD.
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Key Words: ALT, Carotid atherosclerosis, Nonalcoholic fatty liver disease, Metabolic syndrome

Advanced liver diseases which are closely related with metabolic disorders, may stem from simple fatty liver.[1] Fatty liver, the most frequent liver disorder, means an accumulation of fat inside the hepatocytes exceeding 5% of

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Address for correspondence: Seul-Ki Jeong, MD, PhD
Department of Neurology and Research Institute of Clinical Medicine Chonbuk National University-Biomedical Research Institute of Chonbuk National University Hospital, 20 Geonji-ro, Deokjin-gu, Jeonju 561-712, Korea
Tel: +82-63-250-1590, Fax: +82-63-251-9363, E-mail: jeongsk@jbnu.ac.kr

*The authors contributed equally to this manuscript.

the weight of liver. If parameters like alcohol consumption (usually ≥20 g/day), viral hepatitis, and any liver diseases are excluded, nonalcoholic fatty liver disease (NAFLD) is considered for a diagnosis.[2] NAFLD was pathophysiologically related to an inability of insulin to decrease hepatic glucose production, i.e., hepatic insulin resistance.[3] A recent study focused on the independent association between NAFLD and cardiovascular disease (CVD) as well as vascular mortality beyond the relationship of the NAFLD with the hepatic disease itself.[4]

Alanine aminotransferase (ALT), the liver-specific enzyme, was reported to be independently related with mortality from CVD and type 2 diabetes mellitus (DM).[5] With the 10-year follow-up, the Hoorn study reported that subjects in the upper tertile of ALT showed two times higher all-cause mortality, CVD events compared to those in the lower tertile.[6] Bellentani et al.[7] suggested the need of screening for common vascular risk factors if patients showed an increased ALT or NAFLD.

ALT has been reported to be associated with some atherosclerotic measures, but it was limited in subjects with type 2 DM or rheumatoid arthritis,[8,9] but rarely in general population. Practically, it is not easy to interpret ALT as guidance for monitoring of vascular diseases. In the present study, we aimed to examine whether ALT could be associated with carotid atherosclerosis including intima-media thickness (IMT) and plaque in the general population. Because the relation between ALT and atherosclerosis could be obscured if clinical backgrounds were not treated carefully, we selected population who were eligible to diagnose NAFLD.

METHODS

The present cross-sectional study was intentionally designed and executed to reveal relations among ALT, NAFLD, and the metabolic syndrome. Detailed explanations about the design were reported previously.[10,11] In brief, 334 hospital workers were randomly selected according to age- and sex-stratified random cluster samplings, and 135 men and 158 women among them (response 87.7%) participated in the present study. All participants gave their written informed consents to participation in the study, which was approved by the appropriate research ethics committee at the author's institution.

1. Data collection and measurements

The administered questionnaire was designed to determine accurate prior histories of CVD, type 2 DM, hypertension, and medication usage. Information regarding alcohol-drinking status was obtained in terms of the frequency, duration, amount and kind of liquor consumed, from which the mean ethanol intake per day was calculated. Smoking status was classified into three categories: current smokers, ex-smokers, and non-smokers. Waist circumference was measured with subjects standing and wearing no underwear at the level midway between lower rib margin and iliac crest. Body mass index (BMI) was calculated using the usual method, which is weight divided by height squared (kg/m^2).

2. Abdominal and carotid ultrasonography

All abdominal ultrasonographic scans were performed by one radiologist (YKK) who was blinded to the patients' histories and laboratory results. Fatty liver measurements were made in all subjects using a 3.5-MHz convex probe (Sequoia, Siemens Medical Solutions, Mountain View, CA). Hepatic steatosis was diagnosed by characteristic echo-patterns, according to the conventional criteria (i.e., evidence of a diffuse increase in echogenicity of liver as compared with that of the kidney).[12] Repeated measurements on same subjects (28 men and 25 women) gave a coefficient of variation (CV) of less than 1%.

In order to measure the carotid intima-media thickness (IMT), a higher-frequency 7.0-MHz linear transducer (Sequoia; Siemens Medical Solutions, Mountain View, CA) was used with compound and harmonic imaging to reduce near-field artifacts.[13] The carotid IMTs were determined by a double-line pattern visualized by echotomography on the far wall along distal common carotid arteries, carotid bulb, and internal carotid arteries, and measured at six different segments in a region free of plaque. The maximal and mean values obtained in the six segments were used for the present analysis. Atherosclerotic plaque was defined as a focal structure encroaching an arterial lumen by at least 0.5 mm or 50% of the surrounding IMT values, or as the structure where the thickness measured from the media-adventitia interface to the intima-lumen interface was greater than 1.5 mm.[14] Repeated measurements on same objects (30 subjects) gave a CV less

than 4.2%.

3. The metabolic syndrome

The metabolic syndrome was identified by the presence of three or more of the following five components, according to the modified criteria of the Third Adults Treatment Panel (modified ATP-III), with waist cutoffs appropriate for Asian population:[15] 1) abdominal obesity (WC ≥90 cm for men and ≥80 cm for women); 2) high blood pressure (BP) (≥130/85 mmHg or use of antihypertensives); 3) high triglyceride (TG) (≥150 mg/dL); 4) low high-density lipoprotein cholesterol (HDL-C) (<40 mg/dL for men and <50 mg/dL for women); and 5) high fasting glucose (≥100 mg/dL).

4. Biochemical investigations

Blood samples were collected in the morning before breakfast after an overnight fast. Serum biochemistries were assessed with a Hitachi 7600-110 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Most laboratory investigations were described previously in detail.[10] In brief, serum ALT activities were measured according to the International Federation of Clinical Chemistry (IFCC) reference method (ASAN Pharmaceutical Co., Ltd, Korea). Serum insulin and the serological markers of hepatitis B (HBsAg and anti-HBs) and hepatitis C (anit-HCV) were determined by electrochemiluminescence immunoassay (MODULAR ANALYTICS E170, Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance was calculated by homeostasis model assessment for insulin resistance (HOMA-IR) score.[16]

5. Inclusion criteria: NAFLD-eligibility

The criteria for NAFLD-eligibility were based upon the previous report:[2] 1) an alcohol intake of less than 20 g/d, 2) no seropositivity for hepatitis B surface antigen (HBsAg), 3) no seropositivity for anti-hepatitis C virus antibody (anti-HCV), 4) no prior medication history that could cause steatosis, including steroids, amiodarone, estrogens and so on, and 5) no prior history of any kinds of liver disease or elevated iron studies. With the above criteria, the present study selected participants who satisfied all the criteria and excluded those who did not meet any of the criteria.

6. Statistical analysis

The descriptive data for the major characteristics are expressed as the mean±standard deviation (SD) or percentage as appropriately. An independent *t*-test was used to determine the statistical differences in the continuous variables, and a chi-square test for categorical variables. The associations between ALT and carotid IMT or plaque were analyzed with regression methods. Receiver operating characteristic (ROC) curves were used to determine the validity of ALT graphically, and the areas under ROC curves (AUC) were calculated and described. All statistical analyses were conducted using Stata 8.2 software (Stata, College Station, TX).

RESULTS

Among the 293 participants in the present study, 65 (22.2%) subjects who were not eligible for a diagnosis of NAFLD, and 12 (4.1%) subjects who did not undergo either abdominal or carotid ultrasonographic examinations were excluded. Finally, 216 (73.7%, 73 men and 143 women) subjects were analyzed, as shown in Table 1. Age, low-density lipoprotein (LDL) cholesterol, and HOMA-IR did not show significant differences between men and women. However, BMI, ALT, hematocrit,

Table 1. Baseline characteristics

Number	216
Age (years)	43.7±7.2
Women (%)	66.2
Smoking (ex- and current, %)	25.7
Body mass index (kg/m^2)	23.0±2.7
ALT (IU/L)	21.3±12.6
LDL-cholesterol (mg/dL)	110.8±24.7
Hematocrit (%)	40.4±4.1
HOMA-IR	1.5±1.6
Fatty liver (%)	41.7
Metabolic syndrome (%)	11.1
Carotid atherosclerosis	
Carotid maximal IMT (mm)	0.96±0.36
Carotid mean IMT (mm)	0.73±0.10
Carotid plaque (%)	16.7

Values are presented as mean ± SD, unless noted otherwise.
ALT; alanine aminotransferase, LDL; low density lipoprotein, HOMA-IR; homeostasis model assessment-insulin resistance, NAFLD; nonalcoholic fatty liver disease, IMT; intima-media thickness.

Table 2. Prevalence of carotid plaque according to the presence of NAFLD or the metabolic syndrome in each group

	Carotid plaque		*p*
	Absent	Present	
NAFLD			
No (n=126)	110 (87.3%)	16 (12.7%)	0.064
Yes (n=90)	70 (77.8%)	20 (22.2%)	
Metabolic syndrome			
No (n=180)	158 (87.8%)	22 (12.2%)	<0.001
Yes (n=36)	22 (61.1%)	14 (38.9%)	

Numbers (%) were presented.

Table 3. Linear regression analyses between ALT and carotid maximal IMT

(per 1 IU/L)	Carotid maximal IMT (mm)			
	Beta	95% CI	*p*	R^2 (adjusted)
ALT (crude)	0.009	0.005, 0.012	<0.001	0.09
ALT (model 1)	0.008	0.004, 0.011	<0.001	0.20
ALT (model 2)	0.007	0.003, 0.011	0.001	0.22
ALT (model 3)	0.007	0.002, 0.011	0.002	0.27

Model 1; Included age and sex, Model 2; Included variables in model 1 plus hepatic steatosis, the metabolic syndrome, smoking and BMI, Model 3; Included variables in model 2 plus LDL-cholesterol, hematocrit, and HOMA-IR, hypertension, type 2 DM, and dyslipidemia.

Table 4. Logistic regression analyses between ALT and carotid plaque

(per 1 IU/L)	Presence of carotid plaque		*p*
	Odds Ratio	95% CI	
ALT (crude)	1.05	1.02-1.08	<0.001
ALT (model 1)[a]	1.05	1.02-1.08	0.002
ALT (model 2)[b]	1.04	1.10-1.08	0.007
ALT (model 3)[c]	1.05	1.01-1.09	0.026

Model 1; Included age and sex, Model 2; Included variables in model 1 plus hepatic steatosis, the metabolic syndrome, smoking and BMI, Model 3; Included variables in model 2 plus LDL-cholesterol, hematocrit, and HOMA-IR, hypertension, type 2 DM, and dyslipidemia.

the proportions of smokers, NAFLD, and metabolic syndrome were significantly higher in men than women. Carotid mean IMT was significantly higher in men than women, but carotid plaque did not show significant difference.

The proportions of carotid plaque were significantly higher in the participants with the metabolic syndrome, but among those with NAFLD, it reached borderline significance, as shown in Table 2. ALT showed an independent association with carotid maximal IMT, even adjusted for age, sex, and all the possible confounders, as shown in Table 3. ALT also showed an independent and significant association with carotid plaque, even adjusted for the potential confounders. A 1 unit (IU/L) increase of ALT for carotid plaque showed an odd ratio of 1.05 and 95% confidence intervals (CI) as 1.01-1.09, as shown in Table 4. In the excluded subjects, ALT did not show any significant associations with either carotid IMT or plaque (data not shown).

The AUC for carotid plaque of ALT showed 0.69 (95% CI, 0.59-0.78), which was significantly higher than that of NAFLD (0.58, 95% CI; 0.49-0.67, p=0.032) as in Fig. 1. The AUC for carotid plaque of the metabolic syndrome was 0.63 (0.55-0.72), and there was no significant difference between ALT and the metabolic syndrome (p=0.371).

DISCUSSION

The present study demonstrated that ALT (activity) could be directly associated with both carotid IMT and plaque in

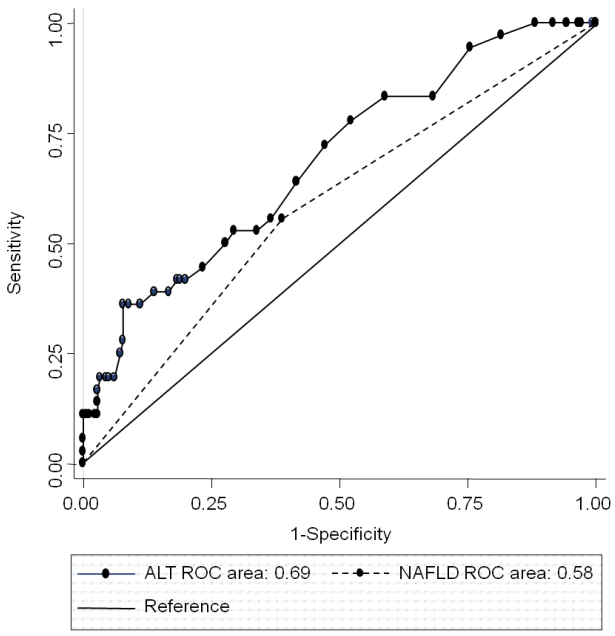

Fig. 1. Receiver operating characteristic (ROC) curves of ALT and NAFLD for carotid plaque. ALT (straight line), NAFLD (dotted line), and diagonal line. Area under ROC curves (AUC): ALT=0.69, NAFLD=0.58, p=0.032.

general population, although the present analysis was performed in the selected population according to the NAFLD-eligibility. Although ALT was a liver-specific enzyme, the association between ALT and carotid atherosclerosis was independent even adjusted for NAFLD or the metabolic syndrome. The inclusion criteria of NAFLD-eligibility appears to be logical to examine the association between ALT and carotid atherosclerosis because ALT can be influenced by variables including alcohol and viral pathogens.

The normally distributed ALT, less than 40 IU/L for men and 30 IU/L for women, showed an independent and graded association with the metabolic syndrome, as given in the authors' previous report[17] and other's long-term follow-up study.[18] Even there were reports on the predictive values of ALT for subsequent vascular events,[6] there seemed to be important confounding effects when the clinical background was not considered carefully.[19] Actually, the robust results of the previous studies were obtained after an exclusion of the subjects with the clinical conditions such as moderate alcohol consumption or viral hepatitis.[4,5,9]

A relation between ALT and hepatic insulin resistance might present some clues for the association between ALT and carotid atherosclerosis. In previous clinical trials with insulin sensitizers, ALT was monitored and reported to be closely related with the improvement of steatohepatitis and its relevant hepatic insulin resistance.[20,21] For example, metformin, an AMP-activated protein kinase activator which improved the sensitivity of glucose uptake to insulin by stimulating the phosphatidylinositol-3-kinase/protein kinase B signaling pathway,[22] was found to successfully lower ALT values in advanced NAFLD patients.[21] In experimental studies, hepatic fat accumulation was found to induce serine phosphorylation of insulin receptor substrate-1 and endoplasmic reticulum (ER) stress, as previously reported.[23] The ER stress, in turn, led to the suppression of insulin receptor signaling, causing hepatic insulin resistance, which could occur primarily[24] and more evidently[25] than peripheral insulin resistance. In addition, the ER stress was also reported to induce CYP2A5 protein increase that caused ALT elevation.[26] The close relationship between ALT and hepatic insulin resistance supported in part the association between ALT and carotid atherosclerosis.

The criteria for NAFLD-eligibility including alcohol drinking and viral hepatitis[2] could be used for subsequent analyses in the stage of grouping itself. Alcohol can modulate both apoptotic and fat synthetic gene expression and subsequently affects liver enzymes diversely.[27] Viral pathogens like hepatitis B virus furthermore could directly affect the progress of carotid atherosclerosis,[28] in addition to their impact on hepatocyte.

The present study has some important limitations. First, ALT was not compared for a conventional measurement for hepatic insulin resistance, like an euglycemic-hyperinsulinemic clamp method. Although the present study for NAFLD was intentionally designed, such a difficult measurement was not selected due to its invasiveness in the present study. Second, the present result came from a cross-sectional design with a relatively small sample size. Thus a subsequent longitudinal study may be warranted to establish a biologic plausibility. Currently, a new study is underway to perform a chylomicron-clearance test for the same subjects involved in the present study. Third, the diet style was not checked with individual basis before 8 hours of fasting, a variable which can affect the outcome of the study because fast food or hyperalimentation could affect ALT profoundly.[29] The present robust results, however, indicate that ALT after 8 hours of fasting could be used appropriately for this kind of analysis.

The present study demonstrated that ALT was significantly

and independently associated with both carotid IMT and plaque in the general population who are eligible for a diagnosis of NAFLD. Subsequent studies are needed to reveal the repeatability of the present findings in the various groups and the direct relation between ALT and hepatic insulin resistance.

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