

Association Between Serum Apolipoprotein A1 Levels, Ischemic Stroke Subtypes and Plaque Properties of the Carotid Artery

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Abstract

Background: This study aimed to investigate the association between serum apolipoprotein A1 (ApoA1) levels, ischemic stroke subtypes and plaque properties.

Methods: We enrolled 92 patients with ischemic stroke and 21 age-matched controls (CONT). The stroke patients were divided into three subtypes: cardioembolic (CE, n = 15), atherothrombotic infraction (ATBI, n = 52), and lacunar infarction (LI, n = 25). Carotid plaques were classified as low, intermediate, or high intensity, and either simple or mixed type. Serum lipids (total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG)), ApoA1, and ApoB were analyzed using commercially available kits.

Results: There was no difference in TC, LDL-C, HDL-C, and ApoB levels among the four groups. Serum ApoA1 levels in the ATBI group were significantly lower compared with the CONT group. Among the ATBI group, the serum ApoA1 levels in the low-intensity plaque-type were significantly lower than those in the intermediate or hard-intensity plaque-type. Furthermore, serum ApoA1 levels in the mixed plaque-type were significantly lower than those in the simple type.

Conclusions: These findings suggest that serum ApoA1 levels might be associated with the development of ATBI and plaque properties of the carotid artery.

Keywords: ApoA1; ATBI; Plaque

Introduction

Stroke is the most prevalent cardiovascular-related condition in Japan. The Hisayama study was started as a population-based prospective cohort study of cerebrovascular and cardiovascular diseases in 1961 in the town of Hisayama. Most of the deceased subjects of the study underwent an autopsy examination. Changes in stroke trends in the last 50 years were clarified by comparing the data from different study cohorts registered every 13 to 14 years. The Suita study was based on a random sampling of Japanese urban residents. Several reports from this study showed the significance of pre-hypertension as well as hypertension as a risk factor for stroke alone and in combination with other underlying characteristics. Stroke is a leading cause of functional dependence and death [1]. The intima-media thickness (IMT) can be quantified using conventional echography. A number of studies have described the relationship between IMT and the risk of myocardial infarction and stroke [2, 3].

Apolipoprotein A1 (ApoA1) plays a major role in reverse cholesterol transport, while apolipoprotein B (ApoB) contributes to the accumulation of cholesterol in plaque [4, 5]. A meta-analysis suggested that reduced ApoA1 levels, increased ApoB levels, and an increased ApoB/A1 ratio are risk factors of ischemic but not hemorrhagic stroke. Elevated ApoA1 levels may be a risk factor of hemorrhagic stroke [6]. ApoA1 and paraoxonase-1 levels may be clinically useful for the diagnosis of ischemic stroke and for differentiating between ischemic and hemorrhagic strokes [7]. However, the association between ApoA1 levels and plaque properties in patients with atherothrombotic infraction (ATBI) is not clear. This study aimed to investigate the association between serum ApoA1 levels and the characteristics of carotid plaque on ultrasonography among the ischemic stroke subtypes.

Materials and Methods

Study design and patient population

We prospectively enrolled 92 patients with stroke and 21 age-matched controls (CONT) between January 1, 2017, and June

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Table 1. Clinical Characteristics of the Participants in the ATBI, CE, LI, and Control Groups

	ATBI (n = 52)	CE (n = 15)	LI (n = 25)	CONT (n = 21)
Age, years	69.7 ± 10.4	66.0 ± 12.3	71.9 ± 9.8	67.5 ± 8.0
Female, %	55.8	60.0	68.0	38.1
BMI, kg/m ²	23.5 ± 3.3	22.5 ± 3.6	22.1 ± 2.6	23.4 ± 3.5
SBP, mm Hg	131.7 ± 17.9	127.5 ± 17.6	134.7 ± 21.4	132.7 ± 13.5
DBP, mm Hg	73.3 ± 11.6	75.3 ± 8.3	72.6 ± 12.2	74.4 ± 11.9
Current smoker, %	33.3	20.0	28.0	14.3
Current alcohol drinker, %	37.3	53.3	36.0	19.0
Hypertension, %	94.2 ^b	60.0	80.0	81.0
Diabetes, %	34.6	6.7	28.0	20.0
Hyperlipidemia, %	78.8 ^c	60.0 ^c	96.0 ^a	61.9
Atrial fibrillation, %	7.7 ^b	100.0 ^{a, c}	4.0	4.8
Statin, %	84.6 ^c	53.8	50.0	66.7

^aP < 0.05, vs. CONT, ^bP < 0.05, vs. CE, ^cP < 0.05, vs. LI. ATBI: atherothrombotic infarction; CE: cardioembolic infarction; LI: lacuna infarction; CONT: control; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

30, 2018, at the National Hospital Organization Kyoto Medical Center in Kyoto, Japan (Table 1). Brain infarction was classified into at least three different stroke subtypes: ATBI, cardioembolic infarction (CE), and lacunar infarction (LI) [8]. The inclusion and exclusion criteria in age-matched controls were 20 years of or older and visiting Neurology Clinics at our hospitals. Exclusion criteria include patients without informed consent agreement. Medical history and laboratory data were obtained from patient's medical records. Diabetes was defined according to the Japan Diabetes Society [9]. Weekly alcohol intake in units of "go" (a traditional Japanese unit of volume corresponding to 23 g of ethanol) was obtained from patients, which was then converted to grams of ethanol per day. One go is 180 mL of sake and corresponds to 1 bottle (633 mL) of beer, two single shots (75 mL) of whiskey, or two glasses (180 mL) of wine. Participants consuming more than 0.3 go per week were regarded as current alcohol drinkers [10]. The study was approved by the Kyoto Medical Center Ethics Committee, Japan (approval number: 18-084). The study protocol conformed to the ethical guidelines of the 2013 Declaration of Helsinki.

Ultrasound evaluation

High-resolution B-mode ultrasonography was performed by trained sonographers using a 7.5-MHz linear array ultrasound imaging system (SSD-2000, Aloka Co. Ltd., Tokyo, Japan). The left and right carotid arteries were scanned with the beam focused on the near and far walls of the distal 2 cm of the common carotid artery proximal to its bifurcation. Both longitudinal and transverse images were obtained to assess the plaque. IMT at the common carotid artery (CCA), carotid bifurcation (BIF), and the internal carotid artery (ICA) were measured. All measurements were made using electronic calipers. The plaque was classified into three categories: 1) Soft plaque-le-

sion echogenicity less than that of the surrounding adventitia for more than 80% of the plaque area without calcium; 2) Hard plaque-lesion echogenicity was greater than or equal to that of the surrounding adventitia for more than 80% of the plaque area without calcium; 3) Intermediate plaque [11]. Significant stenosis was defined as a diameter reduction of > 80%. The end-diastolic velocity (EDV), peak systolic velocity (PSV), mean velocity (mean V), and pulsatility index (PI) were measured.

Laboratory data

Blood samples were collected, and total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and lipoprotein(a) (Lp(a)) levels were measured. Serum levels of ApoA1, A2, B, C2, C3, and E were measured at BML, Inc. (Tokyo, Japan). ApoA1 was evaluated by turbidimetric immunoassay using a commercially available kit (ApoA-I Auto-N' Daiichi', Sekisui Medical Co., Ltd., Tokyo, Japan).

Statistical analyses

All analyses were performed using SSPS for Windows (ver. 24.0; SSPS Inc., Chicago, IL, USA). Continuous variables are presented as the mean ± standard deviation (SD) and categorical variables as counts and percentages. Pearson's Chi-square test was used to compare continuous variables, and one-way factorial analysis of variance (ANOVA) was used for categorical data after testing the normality of the data. Then, the differences were analyzed using *post hoc* Tukey-HSD or Games-Howell multiple comparison tests depending on the results of the assumption of the homogeneity of variances (Levene test). A P value of < 0.05 was considered

Table 2. Serum Lipids and Apolipoproteins of the Participants in the ATBI, CE, LI, and Control Groups

	ATBI (n = 52)	CE (n = 15)	LI (n = 25)	CONT (n = 21)
Serum lipids				
TC, mg/dL	178.4 ± 43.0	195.3 ± 35.8	195.6 ± 55.4	205.6 ± 29.7
LDL-C, mg/dL	106.8 ± 29.7	113.1 ± 27.1	121.0 ± 39.6	118.6 ± 32.2
MDA-LDL, U/L	103.8 ± 44.7	130.7 ± 48.9	100.8 ± 45.1	92.4 ± 18.5
HDL-C, mg/dL	54.6 ± 12.5	57.5 ± 19.4	66.2 ± 17.1	67.0 ± 23.7
TG, mg/dL	147.9 ± 84.2	161.7 ± 107.9	128.5 ± 82.2	142.5 ± 62.9
RLP-C, mg/dL	6.7 ± 7.0	10.0 ± 7.5	7.1 ± 5.1	6.8 ± 3.2
Lp(a), mg/dL	18.0 ± 21.7	21.3 ± 33.5	29.1 ± 44.2	14.8 ± 12.0
Apolipoproteins				
ApoA1, mg/dL	128.6 ± 19.6 ^a	131.9 ± 25.3	139.2 ± 17.1	149.3 ± 21.5
ApoA2, mg/dL	27.9 ± 4.9	30.2 ± 5.5	27.4 ± 4.3	30.1 ± 4.7
ApoB, mg/dL	79.1 ± 17.3	89.2 ± 16.6	80.6 ± 29.1	76.5 ± 9.2
ApoC2, mg/dL	4.5 ± 1.7	5.9 ± 2.3	4.7 ± 2.4	4.9 ± 1.7
ApoC3, mg/dL	9.9 ± 4.3	11.2 ± 3.5	9.9 ± 3.4	10.7 ± 3.0
ApoE, mg/dL	3.1 ± 1.2	3.7 ± 1.2	3.4 ± 1.3	3.4 ± 0.8
HbA1c, %	6.6 ± 1.5	6.0 ± 1.8	6.4 ± 1.3	6.1 ± 0.7

^aP < 0.05, vs. CONT. ATBI: atherothrombotic infarction; CE: cardioembolic infarction; LI: lacuna infarction; CONT: control; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; MDA-LDL: malondialdehyde-modified low-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; RLP-C: remnant lipoprotein cholesterol; Lp(a): lipoprotein(a); ApoA1: apolipoprotein A1; ApoA2: apolipoprotein A2; ApoB: apolipoprotein B; ApoC1: apolipoprotein C1; ApoC2: apolipoprotein C2; ApoE: apolipoprotein E; HbA1c: hemoglobin A1c.

statistically significant.

Results

Background characteristics of the control and stroke patients

The background characteristics of the control subjects and stroke patients are summarized in Table 1. There were no differences in the prevalence of hypertension, diabetes mellitus, atrial fibrillation, current alcohol drinking, and current smoking among the four groups.

Blood analysis and ultrasound evaluation

There was no difference in the TC, LDL-C, HDL-C, and ApoB levels among the four groups (Table 2). The serum ApoA1 levels in the ATBI group were significantly lower compared with the CONT group. Among the ATBI group, the serum ApoA1 levels in the low-intensity plaque-type were significantly lower than those in the intermediate or hard-intensity plaques. Furthermore, the serum ApoA1 levels in the soft plaque-type were significantly lower than those in the intermediate type (Table 3). Among the ATBI group, ApoA1 levels in the heterogeneous type were significantly lower than those in the homogeneous type (Table 4).

Discussion

Main findings

We found that the ApoA1 levels in the ATBI group were significantly lower than those in the CONT group, although there was no difference in the HDL-C levels between the groups.

ApoA1 is the primary lipoprotein associated with HDL in plasma [12]. Lower ApoA1 levels have been found to be associated with a higher stroke risk [13]. In metabolic syndrome, lower levels of HDL-C and ApoA1 were associated with carotid plaques [14]. Our findings support the results of these prior studies. The mechanism of ATBI caused by reduced ApoA1 levels is unknown. The concentration of ApoA1 in the artery wall is thought to enhance cellular cholesterol efflux and protect against atherosclerosis. ApoA1 production by macrophages in the arterial wall is protective against atherosclerosis in mice [15]. Furthermore, ApoA1 levels were associated with plaque properties in the ATBI group. Contrast-enhanced ultrasonography of the carotid artery is a relatively novel diagnostic tool that exploits resonated ultrasound waves from circulating microbubbles. This property permits vascular visualization by producing superior angiography-like images and allows the identification of *vasa vasorum* and intraplaque microvessels [16]. Further examination, including contrast-enhanced ultrasonography, is needed to clarify the mechanism.

Table 3. Ultrasound Evaluation of the ATBI, CE, LI, and Control Groups

Variables	ATBI	CE	LI	CONT
CCA, number of plaques	1.5 ± 1.5 ^{a, b, c}	0.0 ± 0.0	0.3 ± 1.0	0.2 ± 0.7
CCA plaque thickness, mm	2.2 ± 1.1	-	2.9	1.4, 2.0
BIF, number of plaques	2.1 ± 1.3 ^{a, b, c}	0.3 ± 1.0	0.7 ± 1.3	0.3 ± 1.0
BIF plaque thickness, mm	2.2 ± 1.1	2.9	2.2 ± 0.9	1.9, 2.2
ICA, number of plaques	1.7 ± 1.3 ^{a, b, c}	0.1 ± 0.5	0.5 ± 0.9	0.1 ± 0.5
ICA plaque thickness, mm	2.5 ± 1.1	2	2.2 ± 0.9	2.3
Significant stenosis, %	13.7 ^{a, b, c}	0	0	0
PSV, cm/s	62.2 ± 19.4	55.6 ± 10.1	69.8 ± 18.0	65.3 ± 19.6
EDV, cm/s	14.2 ± 5.3	13.2 ± 2.9	13.8 ± 3.5	16.5 ± 5.9
Mean V, cm/s	25.6 ± 7.2	23.9 ± 3.6	28.0 ± 6.3	30.4 ± 8.5
PI	2.4 ± 2.0	1.8 ± 0.4	2.0 ± 0.4	1.7 ± 0.7
Mean IMT of CCA, mm	0.9 ± 0.3	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.2
Plaque category				
Non-plaque, %	5.9 ^{a, b, c}	86.7	62.5	89.5
Soft, %	17.6 ^{a, b, c}	0	0	0
Intermediate, %	39.2 ^{a, b, c}	6.7	16.7	10.5
Hard, %	37.3 ^{a, b, c}	6.7	20.8	0

^aP < 0.05, vs. CONT, ^bP < 0.05, vs. CE, ^cP < 0.05, vs. LI. ATBI: atherothrombotic infraction; CE: cardioembolic infraction; LI: lacuna infraction; CONT: control; CCA: common carotid artery; BIF: carotid bifurcation; ICA: internal carotid artery; EDV: end-diastolic velocity; PSV: peak systolic velocity; mean V: mean velocity; PI: pulsatility index; IMT: intra-media thickness.

Table 4. Serum Lipids and Apolipoproteins Between the Heterogeneous and Homogeneous Type in the ATBI Group

Variables	Heterogeneous type (n = 21)	Homogenous type (n = 27)
Serum lipids		
TC, mg/dL	170.0 ± 23.2 ^a	188.4 ± 39.3
LDL-C, mg/dL	98.0 ± 23.2	113.6 ± 31.6
MDA-LDL, U/L	87.0 ± 33.3 ^a	116.9 ± 50.3
HDL-C, mg/dL	53.6 ± 13.7	54.3 ± 10.9
TG, mg/dL	123.2 ± 43.4	153.7 ± 92.0
RLP-C, mg/dL	7.0 ± 8.9	5.8 ± 4.0
Lp (a), mg/dL	28.3 ± 28.5 ^a	10.6 ± 9.4
Apolipoproteins		
ApoA1, mg/dL	121.0 ± 19.6 ^a	133.8 ± 19.2
ApoA2, mg/dL	26.0 ± 4.2 ^a	29.3 ± 5.4
ApoB, mg/dL	74.7 ± 15.9	82.4 ± 18.4
ApoC2, mg/dL	4.2 ± 1.8	4.7 ± 1.5
ApoC3, mg/dL	8.6 ± 3.4	10.4 ± 4.4
ApoE, mg/dL	2.8 ± 0.9	3.2 ± 1.3
HbA1c, %	6.6 ± 1.3	6.5 ± 1.6

^aP < 0.05, vs. homogenous type. ATBI: atherothrombotic infraction; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; MDA-LDL: malondialdehyde-modified low-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; TG: triglyceride; RLP-C: remnant lipoprotein cholesterol; Lp(a): lipoprotein(a); ApoA1: apolipoprotein A1; ApoA2: apolipoprotein A2; ApoB: apolipoprotein B; ApoC1: apolipoprotein C1; ApoC2: apolipoprotein C2; ApoE: apolipoprotein E; HbA1c: hemoglobin A1c.

Strengths and limitations

The strengths of the present study include that the ultrasound evaluation was undertaken by trained sonographers in a real-world setting. There were several limitations to the present study. First, this was a single-center study with a relatively small sample size. There was no difference in ApoA1 levels between CE and CONT. Careful attention should be paid for interpreting the results, because the smaller sample size in CE (n = 15) compared with the larger sample size in ATBI (n = 52). Second, there are several confounding factors such as age, body mass index (BMI), smoking, and complications. However, we did not analyze controlling for all confounding factors except for age due to the small sample size. Further examination including confounding factors and the large sample are required to clarify these issues in the future. Third, owing to the cross-sectional design, the causal relationship with the determinants could not be ascertained. Fourth, we did not assess the histological characteristics of the carotid plaque. Further examinations, including a histological analysis, are required in the future.

In conclusion, the findings of the current study suggest that serum ApoA1 levels might be associated with the development of ATBI and the plaque properties of the carotid artery.

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Conflict of Interest

None to declare.

Informed Consent

Informed written consents were obtained from the participants.

Author Contributions

RO, SN, and NS conceived and designed the study. SN analyzed the data. RO contributed to participants' data collection. RO, SN, and NS contributed to ethical committee approval. NS wrote the paper.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

References

1. Toyoda K. Epidemiology and registry studies of stroke in Japan. *J Stroke*. 2013;15(1):21-26.
2. Sillesen H, Sartori S, Sandholt B, Baber U, Mehran R, Fuster V. Carotid plaque thickness and carotid plaque burden predict future cardiovascular events in asymptomatic adult Americans. *Eur Heart J Cardiovasc Imaging*. 2018;19(9):1042-1050.
3. de Korte CL, Fekkes S, Nederveen AJ, Manniesing R, Hansen HR. Review: mechanical characterization of carotid arteries and atherosclerotic plaques. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2016;63(10):1613-1623.
4. Panayiotou A, Griffin M, Georgiou N, Bond D, Tylis T, Tziakouri-Shiakalli C, Fessas C, et al. ApoB/ApoA1 ratio and subclinical atherosclerosis. *Int Angiol*. 2008;27(1):74-80.
5. Yatsu FM, Kasturi R, Alam R, Kraus J, Rogers S, DeGraba T. Molecular biology of atherothrombotic brain infarction. *Stroke*. 1990;21(11 Suppl):III131-133.
6. Dong H, Chen W, Wang X, Pi F, Wu Y, Pang S, Xie Y, et al. Apolipoprotein A1, B levels, and their ratio and the risk of a first stroke: a meta-analysis and case-control study. *Metab Brain Dis*. 2015;30(6):1319-1330.
7. Walsh KB, Hart K, Roll S, Sperling M, Unruh D, Davidson WS, Lindsell CJ, et al. Apolipoprotein A-I and paraoxonase-1 are potential blood biomarkers for ischemic stroke diagnosis. *J Stroke Cerebrovasc Dis*. 2016;25(6):1360-1365.
8. Matsuo R, Ago T, Kamouchi M, Kuroda J, Kuwashiro T, Hata J, Sugimori H, et al. Clinical significance of plasma VEGF value in ischemic stroke - research for biomarkers in ischemic stroke (REBIOS) study. *BMC Neurol*. 2013;13:32.
9. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes M, Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Araki E, et al. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig*. 2010;1(5):212-228.
10. Tanaka A, Cui R, Kitamura A, Liu K, Imano H, Yamagishi K, Kiyama M, et al. Heavy alcohol consumption is associated with impaired endothelial function. *J Atheroscler Thromb*. 2016;23(9):1047-1054.
11. Terminology, Diagnostic Criteria Committee JSOUM. Standard method for ultrasound evaluation of carotid artery lesions. *J Med Ultrason* (2001). 2009;36(4):219-226.
12. Walldius G, Jungner I. Apolipoprotein A-I versus HDL cholesterol in the prediction of risk for myocardial infarction and stroke. *Curr Opin Cardiol*. 2007;22(4):359-367.
13. Jungner I, Sniderman AD, Furberg C, Aastveit AH, Hol-

- me I, Walldius G. Does low-density lipoprotein size add to atherogenic particle number in predicting the risk of fatal myocardial infarction? *Am J Cardiol.* 2006;97(7):943-946.
14. Abi-Ayad M, Abbou A, Abi-Ayad FZ, Behadada O, Benyoucef M. HDL-C, ApoA1 and VLDL-TG as biomarkers for the carotid plaque presence in patients with metabolic syndrome. *Diabetes Metab Syndr.* 2018;12(2):175-179.
 15. Major AS, Dove DE, Ishiguro H, Su YR, Brown AM, Liu L, Carter KJ, et al. Increased cholesterol efflux in apolipoprotein AI (ApoAI)-producing macrophages as a mechanism for reduced atherosclerosis in ApoAI((-/-)) mice. *Arterioscler Thromb Vasc Biol.* 2001;21(11):1790-1795.
 16. Filis K, Toufektzian L, Galyfos G, Sigala F, Kourkouveli P, Georgopoulos S, Vavuranakis M, et al. Assessment of the vulnerable carotid atherosclerotic plaque using contrast-enhanced ultrasonography. *Vascular.* 2017;25(3):316-325.