Apolipoproteins B and A1 in Ischemic Stroke Subtypes

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Introduction: Elevated serum apolipoprotein B and the apolipoprotein B/A1 ratio have been associated with ischemic stroke and intracranial atherosclerotic disease. We sought to assess the relationship between serum levels of apolipoprotein B, apolipoprotein A1, and the apolipoprotein B/A1 ratio with ischemic stroke subtypes and large artery atherosclerosis location. *Materials and Methods:* We evaluated serum apolipoprotein B and apolipoprotein A1 levels in consecutive, statin-naïve, adult ischemic stroke patients admitted to an academic medical center in southern India. We evaluated for differences in the mean serum levels of apolipoprotein B, apolipoprotein A1, and the apolipoprotein B/A1 ratio between patients with ischemic stroke attributed to intracranial atherosclerotic disease, extracranial atherosclerotic disease, small vessel disease, and cardioembolism. In secondary analysis, we assessed for differences in these serum apolipoproteins between patients with moderate-severe intracranial atherosclerotic disease and extracranial atherosclerotic disease, irrespective of ischemic stroke subtype. *Results:* Among the 156 ischemic stroke patients enrolled in this study, there were no significant differences in serum levels of apolipoprotein B, apolipoprotein A1, and the apolipoprotein B/A1 ratio between patients with distinct ischemic stroke subtypes. No significant differences were found in serum levels of apolipoprotein B, A1 and the apolipoprotein B/A1 ratio between patients with moderate-severe intracranial atherosclerotic disease and moderate-severe extracranial atherosclerotic disease. *Discussion:* Serum levels of apolipoprotein B and A1 did not differ between ischemic stroke subtypes. Additional studies are needed to validate our findings and to better understand the relationship between serum apolipoproteins and stroke.

Key Words: Ischemic stroke—large artery atherosclerosis—apolipoprotein—stroke subtypes

Introduction

Apolipoproteins (Apo) form the protein component of circulating lipoproteins and function in lipid transport and metabolism. Apo B is a major apolipoprotein component of the pro-atherogenic very low-, intermediate-, and low-density lipoproteins (LDL) whereas Apo A1 is the major apolipoprotein of high-density lipoprotein (HDL). Apo B has been suggested to be a better treatment target for statin therapy compared to LDL and a superior marker of cardiovascular disease risk.[1] INTERSTROKE, an international case-control study, demonstrated that an elevated serum apolipoprotein (Apo) B/A1 ratio was associated with a higher odds of ischemic stroke.[2,3]

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Elevated serum levels of Apo B and the Apo B/A1 ratio have been associated with ischemic stroke in case-control and population-based cohort studies,[4] and have been demonstrated to be predictive of ischemic stroke in transient ischemic attack (TIA) patients.[5] Elevated Apo B/A1 ratio has also been associated with carotid intima-media thickness among patients with cerebral infarction.[6] Furthermore, higher serum Apo B and an elevated Apo B/A1 ratio have been shown to be independent predictors of intracranial atherosclerotic disease (ICAD),[7,8] and have been associated with asymptomatic deep subcortical ischemia in patients with ICAD.[9] The relationship between these apolipoproteins and distinct ischemic stroke subtypes has not been evaluated.

We aimed to evaluate differences in serum levels of Apo B, Apo A1, and the Apo B/A1 ratio in different ischemic stroke subtypes in our primary analysis. We additionally assessed differences in serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between patients with moderate-severe ICAD and extracranial atherosclerotic disease (ECAD), irrespective of ischemic stroke subtype, in our secondary analysis. We hypothesized that serum levels of Apo B and the serum Apo B/A1 ratio would be higher among: 1. patients with ischemic stroke presumed to be secondary to ICAD in comparison to those with other presumed mechanism of cerebral infarction, and; 2. patients with moderate-severe ICAD in comparison to those with moderate-severe ECAD.

Materials and Methods

Study Design and Participants

A cross-sectional study design was utilized to evaluate serum Apo B and Apo A1 levels in consecutive statin-naïve, adult ischemic stroke patients admitted to Sree Chitra Tirunal Institute of Medical Science and Technology, a tertiary care, academic medical center in Trivandrum, Kerala, India. A complete stroke etiologic evaluation was completed in order to determine and classify ischemic stroke etiology by the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria for each enrolled patient.[10] The large artery atherosclerosis subtype of ischemic stroke was further subclassified as ICAD or ECAD, based upon location of presumed atherosclerotic stenosis. The clinical evaluation included neuroimaging (MRI or CT brain), cerebrovascular imaging (MRI angiogram or CT angiogram of the head and neck, carotid and transcranial Doppler ultrasound, or digital subtraction angiography), cardiac assessment (echocardiography, electrocardiogram, cardiac rhythm monitoring), and vascular risk factor screening (serum hemoglobin A1c, total cholesterol, triglycerides, HDL, and LDL). The treating physician determined the presumed ischemic stroke subtype.

We included statin-naive, adult (age $\geq$ 18 years) ischemic stroke patients whose stroke was presumed to be secondary to large artery atherosclerosis, Small Vessel Disease (SVD), or Cardioembolism (CE). We excluded patients whose stroke was due to another determined cause per the TOAST criteria (dissection, vasculitis, hypercoagulable state, etc.) or was cryptogenic. Patients with a history of any previous statin use, prior stroke, and those with intracerebral or subarachnoid hemorrhage were also excluded.

In primary analysis, we compared mean serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between ischemic stroke subtypes. We additionally categorized patients based on the presence of moderate-severe ICAD, ECAD, and both ICAD and ECAD on cerebrovascular imaging, irrespective of ischemic stroke subtype. Moderate-severe ICAD was defined by the presence of greater than or equal to 50% arterial stenosis (asymptomatic or symptomatic) attributed to atherosclerosis on cerebrovascular imaging involving the intracranial portion of internal carotid artery, middle cerebral artery, anterior cerebral artery, posterior cerebral artery, basilar artery, or the intracranial portion of the vertebral artery. Moderate-severe ECAD was defined by the presence of greater than or equal to 50% arterial stenosis (asymptomatic or symptomatic) attributed to atherosclerosis on cerebrovascular imaging involving the extracranial portion of the internal carotid artery or vertebral artery. In secondary analysis, we compared mean serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between patients with moderate-severe ICAD and moderate-severe ECAD; those with concurrent greater than or equal to 50% arterial stenosis from both ICAD and ECAD were excluded from this analysis.

This study was approved by the institutional ethics committee at Sree Chitra Tirunal Institute for Medical Sciences and Technology.

Biochemical Analysis

Serum samples were obtained after a 12-hour period of overnight fasting for the measurement of Apo B, Apo A1, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides within 7 days of the index ischemic stroke. Apo B and A1 were measured by immunoturbidimetry using the Roche Cobas C Analyzer (Roche Diagnostics, Indianapolis, USA) and the Apo B/A1 ratio was calculated. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured using enzymatic methods with the Dimension RxL Max Integrated Chemistry System (Siemens Healthcare Diagnostics Inc., Newark, NJ).

Statistical Analyses

Patient characteristics and lipid measurements were summarized as means/standard deviations or medians/interquartile ranges for continuous variables and proportions for categorical variables. Differences in patient characteristics between ischemic stroke subtypes were assessed

using one-way analysis of variance for continuous variables and χ^2 analysis for categorical variables. For our primary analysis, differences in lipid measurements between ischemic stroke subtypes were assessed utilizing one-way analysis of variance. For our secondary analysis, differences in lipid measurements between patients with moderate-severe ICAD and ECAD, irrespective of ischemic stroke subtype, were compared using 2-tailed student's *t*-tests. A *P*-value less than .05 was considered significant. All analyses were conducted using R 3.5.1.

Results

Among the 156 ischemic stroke patients enrolled, the TOAST classification of the index ischemic strokes were 34 ICAD, 27 ECAD, 55 SVD, and 40 CE. The vascular imaging modality used for assessment of ICAD and ECAD was ultrasound for 23 patients, CT angiography for 107, MR angiography for 60, and digital subtraction angiography for 9; more than 1 diagnostic imaging modality was utilized for 38 patients. Irrespective of ischemic stroke subtypes, 53 patients had asymptomatic or symptomatic moderate-severe ICAD, 22 had moderate-severe ECAD, 8 had both moderate-severe ICAD and ECAD, and 73 had no evident atherosclerotic stenosis of the intracranial or extracranial arteries. Baseline demographics, vascular risk factors, and lipid measurements for all enrolled patients and for each ischemic stroke subtype are summarized in Table 1. There were differences in age, gender, and certain vascular risk factors – hypertension, diabetes, hyperlipidemia, atrial fibrillation, and tobacco use – between patients with distinct ischemic stroke subtypes. In our primary analysis, we found no significant differences in serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between patients with cerebral infarction presumed to be secondary to ICAD, ECAD, SVD, or CE (Table 2). In our secondary analysis, irrespective of ischemic stroke subtype, patients with moderate-severe ICAD had higher serum levels of Apo B in comparison to patients with moderate-severe ECAD, although the difference was not statistically significant (117.9 mg/dL versus 97.0 mg/dL, $P = 0.083$). Serum levels of Apo A1 and the Apo B/A1 ratio did not differ between patients with moderate-severe ICAD and ECAD (Table 3).

Discussion

We found no significant differences between serum levels of Apo B, Apo A1, and the Apo B/A1 ratio among ischemic stroke subtypes. However, we found that serum levels of Apo B were nonsignificantly higher among ischemic stroke patients with moderate-severe ICAD in comparison to those with moderate-severe ECAD, irrespective of the presumed mechanism of infarction.

To our knowledge, this is the first report assessing differences in serum Apo B and A1 among ischemic stroke subtypes. Previous cohort and case-control studies have suggested that reduced Apo A1 levels and increased Apo B levels and the Apo B/A1 ratio are risk factors for first ischemic stroke.[4] Although there may be a relationship between these apolipoproteins and overall ischemic stroke risk, our findings suggest that serum levels of Apo B, Apo A1, and the Apo B/A1 ratio may not be associated with a particular mechanism of cerebral infarction. Prior studies have evaluated the relationship between serum levels of these apolipoproteins with ICAD and ECAD. A prior report has shown that the Apo B/A1 ratio was higher among Korean ischemic stroke patients with ICAD in comparison to those with ECAD or no cerebral atherosclerotic stenosis.[8] A dose-response relationship was found between Apo B/A1 ratio quartiles and the burden of ICAD in this population; in multivariable analysis, the highest Apo B/A1 ratio quartile was an independent predictor of ICAD.[8] Another hospital-based study of Chinese ischemic stroke patients demonstrated that elevated serum level of Apo B was an independent risk factor for the presence of ICAD and ECAD; Apo A1 levels were significantly lower among patients with ICAD in this study.[7] In our study, Apo B, Apo A1 and Apo B/A1 ratio was not found to be statistically significant between patients with ICAD and those with ECAD. Genetic, environmental, or comorbidity differences between the East Asian populations from prior reports and the South Asian population from our study may also contribute to differences in the findings. The INTERSTROKE case-control study demonstrated substantial regional variation in the magnitude of the association between the Apo B/A1 ratio and stroke risk.[3] It is possible that these apolipoproteins differ among ischemic stroke subtypes in other populations.

The principal strength of this study is that ischemic stroke subtype classification was meticulously completed as all patients underwent a thorough ischemic stroke etiologic evaluation. However, our study does have notable limitations. This was a single center study in India, which limits the generalizability of the findings to other populations. The analysis was cross-sectional in nature, which limits interpretation about any causal link between apolipoproteins B and A1 with ischemic stroke subtypes or large artery atherosclerosis location. Due to the small number of patients in our study groups, it is possible that our study is underpowered to assess for true differences between serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between ischemic stroke subtypes or by location of large artery atherosclerosis. Finally, there was heterogeneity in the vascular imaging modality used for assessment of large artery atherosclerosis; CT angiography, and MR angiography have good sensitivity and specificity in the identification of moderate-severe large artery atherosclerotic stenosis.[11-13]

In summary, serum levels of Apo B, Apo A1, and the Apo B/A1 ratio were not significantly different between ischemic stroke subtypes among South Asian, statin-naïve, adult ischemic stroke patients or between patients

Table 1. *Baseline characteristics*

	Ischemic stroke patients (n = 156)	Ischemic stroke subtypes per TOAST criteria				
		ICAD (n = 34)	ECAD (n = 27)	SVD (n = 55)	CE (n = 40)	*P*
Age, mean (SD), years	60.1 (12.1)	61.6 (11.7)	62.7 (11.3)	60.5 (10.2)	56.4 (14.7)	.050
Male, n (%)	108 (69.2)	27 (79.4)	23 (85.2)	31 (56.4)	27 (67.5)	.027
BMI, mean (SD)	23.8 (2.2)	24.2 (2.3)	23.2 (2.2)	23.9 (2.3)	23.5 (1.9)	.364
NIHSS, median (IQR)	6 (3-11)	5 (2-12)	8 (4-15)	5 (3-8)	9 (4-14)	.619
Vascular risk factors, n (%)						
Hypertension	102 (65.4)	26 (76.5)	15 (55.6)	42 (76.4)	19 (47.5)	.009
Diabetes	79 (50.6)	21 (61.8)	18 (66.7)	31 (56.4)	9 (22.5)	<.001
Coronary artery disease	13 (8.3)	3 (8.8)	3 (11.1)	1 (1.8)	6 (15.0)	.129
Hyperlipidemia	38 (24.4)	5 (14.7)	8 (29.6)	21 (38.2)	4 (10.0)	.006
Atrial fibrillation	23 (14.7)	1 (2.9)	0 (.0)	0 (.0)	22 (55.0)	<.001
Prior stroke	10 (6.4)	2 (5.9)	1 (3.7)	3 (5.5)	4 (10.0)	.732
Tobacco use	65 (41.7)	18 (52.9)	17 (63.0)	17 (30.9)	13 (32.5)	.013
Alcohol use	47 (30.1)	14 (41.2)	11 (40.7)	13 (49.1)	9 (22.5)	.236
Lipid measurements, mean (SD), mg/dL						
Total cholesterol	199.7 (51.4)	195.0 (44.7)	204.9 (41.9)	210.9 (61.3)	184.7 (44.8)	.549
High-density lipoprotein cholesterol	43.5 (12.4)	40.2 (9.4)	46.1 (14.3)	45.5 (12.5)	41.9 (12.6)	.590
Low-density lipoprotein cholesterol	133.7 (43.1)	127.5 (40.2)	137.7 (34.4)	142.4 (51.3)	124.2 (36.5)	.909
Triglycerides	116.1 (69.9)	123.4 (48.2)	114.1 (60.0)	118.6 (83.4)	107.8 (72.3)	.419

Abbreviations: BMI, body-mass index; ICAD, intracranial atherosclerotic disease; ECAD, extracranial atherosclerotic disease; IQR, interquartile range; mg/dL, milligrams/deciliter; NIHSS, national institutes of health stroke scale; SD, standard deviation; TOAST, Trial of Org 10172 in acute stroke treatment.

P values are from one-way analysis of variance for continuous variables and χ^2 analysis for categorical variables.

Table 2. *Comparison of mean serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between ischemic stroke subtypes (per TOAST criteria)*

	Ischemic stroke subtypes				
	ICAD (n = 34)	ECAD (n = 27)	SVD (n = 55)	CE (n = 40)	*P**
Apolipoprotein B, mean (SD), mg/dL	120.7 (43.6)	104.2 (41.2)	104.3 (45.6)	106.4 (53.3)	.203
Apolipoprotein A1, mean (SD), mg/dL	117.9 (46.7)	108.2 (43.7)	113.0 (48.6)	112.6 (66.5)	.761
Apolipoprotein B/A1 ratio	1.06 (.28)	1.03 (.32)	.97 (.36)	1.03 (.38)	.511

See footnote for Table 1.
**P* values are from one-way analysis of variance.

Table 3. *Comparison of mean serum levels of Apo B, Apo A1, and the Apo B/A1 ratio between patients with evident ICAD and ECAD, irrespective of ischemic stroke subtype*

	ICAD (n = 51)	ECAD (n = 25)	*P**
Apolipoprotein B, mean (SD), mg/dL	117.9 (49.1)	97.0 (47.1)	.083
Apolipoprotein A1, mean (SD), mg/dL	113.2 (49.1)	101.7 (47.1)	.337
Apolipoprotein B/A1 ratio	1.08 (.33)	1.02 (.35)	.492

See footnote for Table 1.
**P* values are from 2-tailed student's t-test.

with moderate-severe ICAD and those with moderate-severe ECAD in this population. Prospective studies and studies conducted in other populations are needed to better understand the relationship between serum apolipoproteins, ICAD, and ischemic stroke.

Conflict of Interest

The authors report no conflicts of interest.

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