PRELIMINARY EVIDENCE ON THE DIAGNOSTIC VALUE OF APOLIPOPROTEIN B AND A-I IN PERIPHERAL ARTERY DISEASE AMONG VIETNAMESE PATIENTS WITH DIABETES

Nguyen Manh Ha[1,✉], Nguyen Thi Ho Lan[1], Pham Thai Binh[1]
Vu Minh Phuc[1], Hoang Van Kien[1], Tran Thi Nhu Quynh[1]
Nguyen Thi Nhu Quynh[1], Nguyen Thi Giang[1]
Nguyen Thi Thu[1], Nguyen Thi Minh Thu[1], Nguyen Ngoc Mai[1]
Nguyen Thi Ngoc Hoa[1], Dinh Thi Thu Huong[2]
[1]Vietnam National Endocrinology Hospital
[2]Hanoi Medical University

To evaluate the value of apolipoprotein A-I, B, and the apoB/AI ratio in the diagnostic of peripheral artery disease among patients with diabetes, we conducted a cross-sectional study of which they underwent clinical evaluation, standardized Doppler ultrasound and blood test. Area under the Receiver Operating Characteristic curve method was employed to estimate the discriminatory ability of apolipoprotein A-I, apolipoprotein B, and the ApoB/A-I ratio. A total of 159 patients were included. ApoA-I, ApoB, and the ApoB/A-I ratio show statistically significant associations with most clinical and Doppler ultrasound characteristics of PAD. In Pearson's correlation analysis, apolipoproteins showed a stronger correlation with ABI than the traditional lipid profile. The AUROC was 0.714 (95%CI: 0.635 - 0.794); 0.300 (95%CI: 0.219 – 0.380) and 0.604 (95%CI: 0.516 – 0.692) for apoB/A-I ratio; apoAI and apoB, respectively. The probability apoB/A-I cutoff of 0.666 had a sensitivity of 0.682 and a specificity of 0.662. Apolipoprotein AI and Apolipoprotein B are tests with considerable potential in diagnosing peripheral arterial disease in patients of type 2 diabetes mellitus patients. Further studies are needed with larger sample sizes, and employing imaging diagnostic modalities with higher reliability as the gold standard (MSCT angiography).

Keywords: PAD, diabetes, apolipoprotein, doppler, cholesterol.

I. INTRODUCTION

The global prevalence of type 2 diabetes mellitus (T2DM) in the age group of 20 - 79 is currently estimated at 10.5% of the global population, equivalent to 536.6 million people. This number is projected to increase to 12.2% of the global population, equivalent to 783.2 million people by the year 2045.[1] T2DM is a major risk factor for the development of atherosclerosis, as well as an increased incidence of disability and mortality associated with cardiovascular diseases.[2] Peripheral arterial disease (PAD) is a condition characterized by localized atherosclerosis resulting in insufficient regional blood supply to the extremities. The presence of PAD is associated with an increased risk of limb amputation and serves as an indicator of atherosclerosis in larger arteries such as the coronary, cerebral, and renal arteries, contributing to an elevated risk of myocardial infarction, stroke, and mortality.[3]

Diabetes management requires a

Corresponding author: Nguyen Manh Ha
Vietnam National Endocrinology Hospital
Email: manhhanoitiet@gmail.com
Received: 10/06/2024
Accepted: 02/07/2024

multifaceted approach, with a specific focus on PAD screening. While recent tools such as ultrasonography and Ankle-Brachial Index (ABI) measurement have been employed for PAD detection, their widespread utilization faces certain limitations. The ABI, for example, is time consuming, relies on precise blood pressure measurements, and in the presence of arterial stiffness common in individuals with diabetes, it may underestimate the severity of PAD. The widespread use of ultrasonography is limited by equipment availability and the need for specialized expertise in cardiology ultrasound, especially in low and middle income countries. Furthermore, the characteristics of PAD in patients with T2DM include diffuse, multi-level, and multi-branch lesions, predominantly affecting small peripheral arteries. This makes it increasingly challenging and time-consuming to assess the degree of vascular stenosis using ultrasound, potentially leading to inaccuracies in evaluation. As such, in certain settings, including primary care and emergency medicine, alternative approaches are required.

Evidence from recent studies has shown that lipid-related proteins, particularly apolipoprotein A-I and B, are more effective indicators than LDL cholesterol and other blood lipid components in predicting the risk of myocardial infarction.[4-6] These apolipoprotein tests are easily accessible, less invasive, and are increasingly recognized for their value in diagnosis, prognosis, and estimating the risk of arterial occlusion. Recent studies indicate that the association between an increased apolipoprotein B/A-I ratio and the risk of arterial occlusion may extend to peripheral arteries, making it a specific indicator for all arterial occlusive events.[7,8] However, to date, there has been no study evaluating the significance of these markers in screening for PAD in T2DM individuals. Therefore, our study aims to fill this gap by evaluating the diagnostic value of apolipoprotein A-I, B, and the apoB/AI ratio in the T2DM population.

II. MATERIALS AND METHODS

1. Subjects

Patient eligibility

Eligible participants included individuals diagnosed with type 2 diabetes mellitus according to the 2022 American Diabetes Association (ADA) criteria, as outlined below: (1) fasting plasma glucose levels ≥ 126 mg/dL (7.0 mmol/L), where fasting is defined as no caloric intake for at least 8 hours; or (2) 2-hour postprandial glucose (2-h PG) levels ≥ 200 mg/dL (11.1 mmol/L) during oral glucose tolerance test, conducted as per the World Health Organization guidelines, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water; or (3) hemoglobin A1C levels ≥ 6.5% (48 mmol/mol), determined in a laboratory using a method certified by the National Glycohemoglobin Standardization Program and standardized to the Diabetes Control and Complications Trial assay; OR (4) In a patient exhibiting classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose level ≥ 200 mg/dL (11.1 mmol/L).

Exclusion criteria included: (1) type 1 diabetes, specific types of diabetes due to other causes and gestational diabetes mellitus; (2) acute lower limb ischemia; (3) stenosis of the lower limb arteries in Takayasu's disease, Buerger's disease, Raynaud's syndrome; (4) other causes of peripheral arterial stenosis (tumors, entrapment syndrome, trauma).

2. Methods

Study design

A cross-sectional study was conducted from August 2022 to July 2023 at the Vietnam

National Heart Institute and National Hospital of Endocrinology in 159 patients diagnosed with type 2 diabetes mellitus.

Data collections

The participants underwent a comprehensive clinical evaluation, which included physical examination, medical history review, standardized Doppler ultrasound and blood test. At the beginning of the study, demographic characteristics, comorbidities, smoking status, current medications for diabetes treatment, diabetes duration and complications were documented. Blood tests included fasting plasma glucose, hemoglobin A1C, total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-I, and apolipoprotein B. Blood glucose control status was assessed based on A1c level, according to the specific recommendations of the American Diabetes Association (ADA) in 2022 as follows: Achieved: < 7.0%; Not achieved: ≥ 7.0%.

The Ankle-Brachial Index serves as a pivotal non-invasive diagnostic tool for the assessment of PAD. According to a standardized protocol, ABI measurements were conducted by a trained physicians of the research team, using Doppler ultrasound technology in conjunction with blood pressure cuffs (LifeDop 250 Series Hand-Held Doppler with 8MHz Vascular Probe). Prior to measurement, patients are positioned supine, allowing for a brief resting period. The Doppler probe is strategically applied to detect arterial signals at the brachial artery, dorsalis pedis, and posterior tibial arteries. Systolic blood pressure readings are then obtained at each location, and the ABI is calculated by dividing the highest ankle pressure by the highest arm pressure. If there is a discrepancy between the values obtained from the left and right sides, the lowest result would be considered to represent the patient's ABI value. The ABI values are subsequently classified based on the following clinical significance: (1) > 1.3: Arterial stiffness/ calcification; (2) 0.9 - 1.3: Normal; (3) 0.7 - 0.9: Mild peripheral arterial disease; 0.4 - 0.7: Moderate peripheral arterial disease; < 0.4: Severe peripheral arterial disease.

Doppler ultrasound was conducted by an independent cardiologist who was blind to the clinical and lipid profile data, at 21 locations: abdominal aorta, common iliac, internal iliac, external iliac, common femoral, deep femoral, superficial femoral, popliteal, anterior tibial, posterior tibial, dorsalis pedis. Stenosis degree was determined as % Stenosis = [1 – (stenotic lumen diameter/post-stenotic arterial segment diameter)] x 100%. Results were categorized using North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria: (0) Normal; (1) Mild stenosis (< 50% vessel diameter); (2) Moderate stenosis (50% - 69% vessel diameter); (3) Severe stenosis (70% - 99% vessel diameter); and (4) Total occlusion.

Based on the Doppler ultrasound results, patients were categorized into one of two groups: with or without PAD, according to the 2016 American Heart Association/American College of Cardiology (AHA/ACC) criteria.[9] This includes ABI < 0.9 and/or Doppler ultrasound revealing stenosis or occlusion of ≥ 50% of the arterial diameter in the lower extremities.

Patients with PAD would be further evaluated regarding PAD duration, medication, signs and symptoms, which included claudication, ischemic rest pain, cold feet, purple discoloration, dry skin, and necrosis. Clinical examination of peripheral arteries was conducted in the femoral artery (within the Scarpa triangle), the popliteal artery (at the popliteal fossa), the anterior tibial artery (at the ankle), and the posterior tibial artery (at the

posterior tibial fossa), and was categorized as symmetrical/pulse present on both sides, or asymmetrical/absent pulse on one or both sides. Based on clinical characteristics, patients were classified according to the Rutherford clinical classification as follows: 0 - no symptoms; 1 - mild claudication; 2 - moderate claudication; 3 - severe claudication; 4 - ischemic rest pain; 5 - minor tissue lost; 6 - major tissue lost.

Statistical analysis

Continuous variables were presented as mean and standard deviation, categorical variables were presented as frequencies and percentages. Horizontal stacked bar charts were utilized to illustrate the distribution of degrees of arterial stenosis in Doppler ultrasound. Spearman's correlation coefficient test and scatter plot were employed to estimate the correlations between ABI and traditional lipid profile/apolipoproteins. Differences between two groups for continuous variables with a normal distribution were assessed using the t-test. For continuous variables without a normal distribution, differences between two groups were assessed using the Mann-Whitney test or Kruskal-Wallis test. Differences for categorical variables were assessed using the Chi-square test or Fisher's exact test. Multivariate logistic regression was employed to adjust for potential confounding factors (including age, gender, comorbid hypertension, smoking status, glycemic control, and LDL cholesterol levels) in assessing the relationship between apolipoproteins and the presence of PAD. AUROC (Area Under the Receiver Operating Characteristic curve) was employed to estimate the discriminatory ability of apolipoprotein A-I, apolipoprotein B, and the ApoB/A-I ratio in distinguishing between patients with and without PAD.

3. Research ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Hanoi Medical University under decision No. 2288/QĐ-ĐHYHN dated July 15th 2023. Informed consent was obtained from all patients before participating in the study. The investigators were responsible for protecting the privacy and confidentiality of patients as per Vietnam's regulations and Good Clinical Practice.

III. RESULTS

A total of 159 patients were included in this study. Compared to the non-PAD group, the PAD group had a higher rate of male patients (78.8% vs. 48.6%), older age (71.34 ± 9.41 vs. 61.46 ± 11.58), lower prevalence of overweight (31.8% vs. 47.3%), and a higher smoking prevalence (77.6% vs. 14.9%). Among the comorbidities, hypertension was the most common (57.9%). The PAD group primarily consisted of patients using oral medication alone (32.7%) and insulin (40.3%), while the non-PAD group mainly included patients using insulin (39.2%) and combined insulin/oral medication (35.1%). In comparison to the non-PAD group, the PAD group had a higher rate of achieving target glycemic control (36.5% vs. 16.2%) and a higher rate of cases without complications (56.5% vs. 24.3%). Claudication, rest ischemic pain and necrosis were observed in 56.5%, 60% and 37.6% of the patients in PAD group, respectively. The majority of patients in the PAD group were classified as Rutherford III (29.4%), IV (24.7%) and V (32.9%), and most of them were using statins (93% in the PAD group and 100% in the non-PAD group). The demographic and clinical characteristics of the participants are presented in Table 1.

Table 1. Demographic and clinical characteristics (n = 159)

Characteristics	Overall (n = 159)	PAD (n = 85)	No PAD (n = 74)	p
Male, n (%)	103 (64.8)	67 (78.8)	36 (48.6)	*< 0.001*
Age (years), mean ± SD	66.74 ± 11.55	71.34 ± 9.41	61.46 ± 11.58	*< 0.001*
BMI group, n (%)				
Underweight	9 (5.7)	9 (10.6)	0	*0.003*
Normal	88 (55.3)	49 (57.6)	39 (52.7)	
Overweight	62 (39.0)	27 (31.8)	35 (47.3)	
Comorbidities, n (%)				
CAD	19 (11.9)	17 (20.0)	2 (2.7)	*0.001*
HF	10 (6.3)	9 (10.6)	1 (1.4)	*0.021*
HTS	92 (57.9)	50 (58.8)	42 (56.8)	0.872
Stroke	11 (6.9)	7 (8.2)	4 (5.4)	0.546
Asthma/COPD	1 (0.6)	0	1 (1.4)	0.465
CKD	9 (5.7)	2 (2.4)	7 (9.5)	0.083
Cancer	4 (2.5)	2 (2.4)	2 (2.7)	1.000
Smoker, n (%)	77 (48.4)	66 (77.6)	11 (14.9)	*< 0.001*
Diabetes duration (months), mean ± SD	90.69 ± 81.76	68.95 ± 62.55	115.65 ± 93.73	*< 0.001*
Diabetes medication, n (%)				
Lifestyle modification	9 (5.7)	1 (1.2)	8 (10.8)	*< 0.001*
Oral medication	52 (32.7)	41 (48.2)	11 (14.9)	
Insulin	64 (40.3)	35 (41.2)	29 (39.2)	
Oral medical plus Insulin	32 (20.1)	6 (7.1)	26 (35.1)	
Others	2 (1.3)	2 (2.4)	0	
No complications, n (%)	66 (41.5)	48 (56.5)	18 (24.3)	*< 0.001*
FPG (mmol/l), mean ± SD	9.99 ± 3.69	9.69 ± 3.29	10.34 ± 4.09	0.267
A1c (%), mean ± SD	8.77 ± 2.06	8.57 ± 1.92	8.99 ± 2.20	0.206
Glycemic control achieved, n (%)	43 (27.0)	31 (36.5)	12 (16.2)	*0.004*
PAD duration (months), mean ± SD	-	3.89 ± 7.92	-	-
PAD signs and symptoms, n (%)				
No symptoms	-	4 (4.7)	-	-
Claudication	-	48 (56.5)	-	-

Characteristics	Overall (n = 159)	PAD (n = 85)	No PAD (n = 74)	p
PAD signs and symptoms, n (%)				
Rest ischemic pain	-	51 (60.0)	-	-
Cold feet	-	40 (47.1)	-	-
Purple discoloration	-	38 (44.7)	-	-
Dry skin	-	43 (50.6)	-	-
Necrosis	-	32 (37.6)	-	-
Asymmetrical/absent pulse				
Femoral artery	-	55 (64.7)	-	-
Popliteal artery	-	55 (64.7)	-	-
Anterior tibial artery	-	60 (70.6)	-	-
Posterior tibial artery	-	60 (70.6)	-	-
PAD medications				
Antiplatelet	-	77 (90.6)	-	-
Statin	-	79 (93.0)	74 (100)	0.735
None	-	6 (7.0)	-	-
ABI group, n (%)				
> 1.3	0	0	0	*< 0.001*
0.9 - 1.3	76 (47.8)	3 (3.5)	73 (98.6)	
0.7 - 0.9	25 (15.7)	24 (28.2)	1 (1.4)	
0.4 - 0.7	44 (27.7)	44 (51.8)	0	
< 0.4	14 (16.5)	14 (16.5)	0	
Rutherford, n (%)				
I	-	0	-	-
II	-	2 (2,4)	-	
III	-	25 (29,4)	-	
IV	-	21 (24,7)	-	
V	-	28 (32,9)	-	
VI	-	9 (10,6)	-	

PAD, peripheral artery disease; BMI, body mass index; CAD, coronary artery disease; HF, heart failure; HTS, hypertension; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; FPG, fasting plasma glucose; TC, total cholesterol; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; TG, triglycerides; ABI, ankle-brachial index; SD, standard deviation. Variables in bold are statistically significant

The arterial stenosis degrees were distributed evenly between the left and right sides, and gradually increased from central to peripheral regions. Specifically, in the pelvic level, the prevalence of mild stenosis, moderate stenosis, and severe stenosis to total occlusion was 10-30%, 3 - 10%, and 0 - 10%, respectively. In the thigh level, these rates were 30 - 60%, 10 - 20%, and 0 - 15%, respectively. In the below-knee level, the rates were 30 - 60%, 10 - 20%, and 5 - 25%, respectively. Degrees of arterial stenosis are presented in Chart 1.

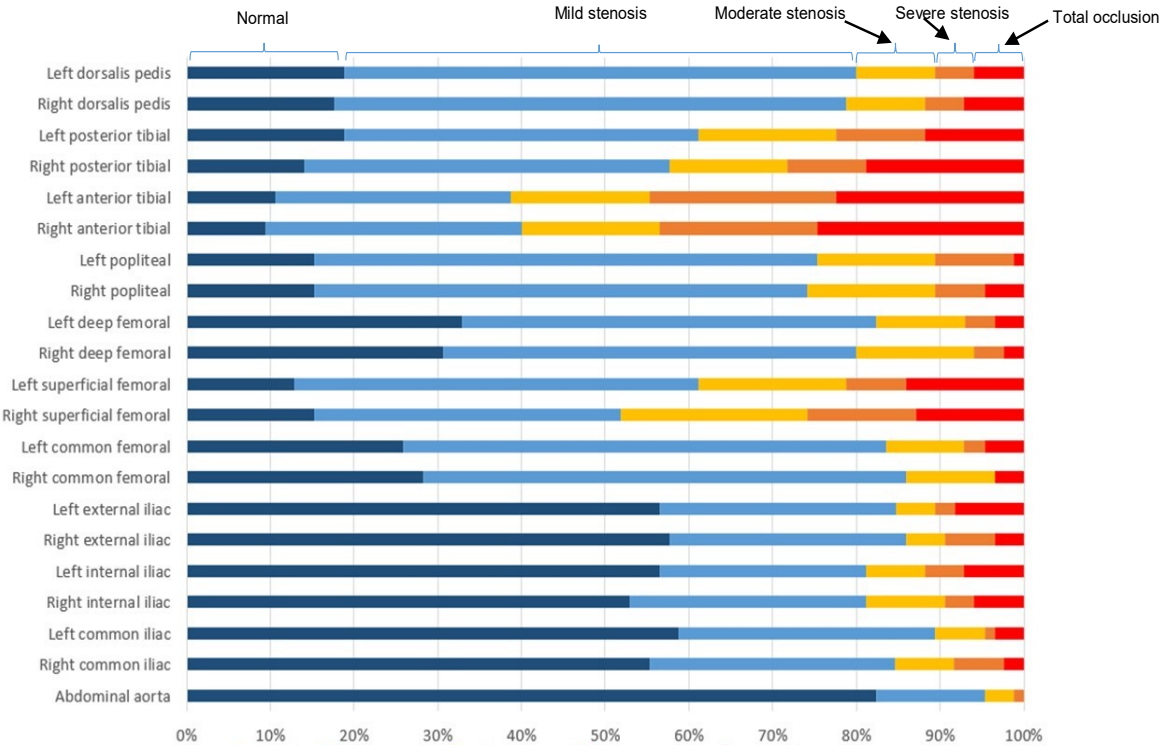

Chart 1. Degrees of arterial stenosis

There are few statistically significant associations between TC, LDLC, HDLC, and TG with the clinical and Doppler ultrasound characteristics of PAD. The mean HDLC level in patients with claudication/rest ischemic pain was lower than that of the asymptomatic group and lower in patients with necrosis than those without necrosis. The mean TC, LDLC, and TG levels in patients with multi-level stenosis were lower than those in the group without multi-level stenosis. Meanwhile, ApoA-I, ApoB, and the ApoB/A-I ratio show statistically significant associations with most clinical and Doppler ultrasound characteristics of PAD. The mean ApoAI level was higher in asymptomatic patients and patients without necrosis, decreasing with the increasing severity of ABI and Rutherford levels. In contrast, ApoB level and ApoB/AI ratio are higher in patients with claudication, ischemic rest pain, and necrosis, increasing with the increasing severity of ABI and Rutherford levels. The mean ApoAI level in patients with stenosis > 50%, total occlusion, and multilevel stenosis was lower; conversely, the mean ApoB level and ApoB/AI ratio in the group with > 50% stenosis, total occlusion, and multilevel stenosis were higher than in the group without these characteristics (Table 2). A similar finding was revealed in Pearson's correlation analysis, where apolipoproteins showed a stronger correlation with ABI than the traditional lipid profile (Chart 2).

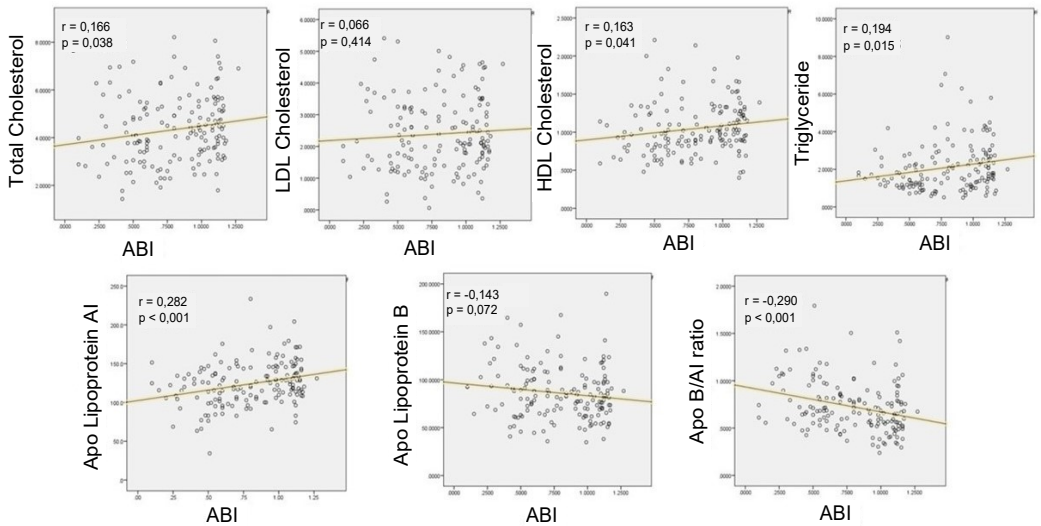

Chart 2. Associations between traditional lipid profile and apolipoproteins with ABI (n = 159)

After adjustment for age, gender, hypertension, smoking status, glycemic control status and LDLC level; apolipoprotein B and apoB/A-I ratio remained as independent factors associated with a higher likelihood of having PAD in patients with diabetes, with OR of 1.028 and 1.027, respectively. After adjustment for age, gender, hypertension, smoking status, glycemic control status and LDLC level; apolipoprotein B and apoB/A-I ratio remained as independent factors associated with a higher likelihood of having PAD in patients with diabetes, with OR of 1.028 and 1.027, respectively. (Table 3)

Table 3. Factors associated with peripheral artery disease in patients with diabetes (n = 159)

	Model 1		**Model 2**		**Model 3**	
Factor	OR	p	OR	p	OR	p
Age	1.190	*< 0.001*	1.208	*< 0.001*	1.194	*< 0.001*
Gender (male vs female)	1.130	0.881	1.286	0.765	1.251	0.787
HTS (yes vs no)	0.336	0.112	0.303	0.088	0.384	0.174
Smoking (yes vs no)	38.523	*< 0.001*	51.225	*< 0.001*	37.910	*< 0.001*
Glycemic control achieved (yes vs no)	1.718	0.380	2.040	0.267	1.794	0.348
LDLC	1.121	0.613	0.704	0.207	0.841	0.475
ApoAI	0.987	0.199	-	-	-	-
ApoB	-	-	1.028	*0.018*	-	-
ApoB/ ApoAI	-	-	-	-	1.027	*0.014*

HTS, hypertension; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; TG, triglycerides; ABI, ankle-brachial index; SD, standard deviation
Variables in bold are statistically significant

Table 2. Associations between traditional lipid profile and apolipoproteins with PAD characteristics (n = 159)

Factor	n	TC (mmol/l)	p	LDLC (mmol/l)	p	HDLC (mmol/l)	p	TG (mmol/l)	p	ApoAI (mg/dL)	p	ApoB (mg/dL)	p	ApoB/A-I	p
		$\bar{x}$ ± SD		$\bar{x}$ ± SD		$\bar{x}$ ± SD		$\bar{x}$ ± SD		$\bar{x}$ ± SD		$\bar{x}$ ± SD		$\bar{x}$ ± SD	
Claudication/Rest ischemic pain															
No	78	4.52 ± 1.25	0.095	2.47 ± 1.06	0.377	1.11 ± 0.31	*0.014*	2.19 ± 1.26	0.401	132.84 ± 24.16	*< 0.001*	81.70 ± 24.96	0.053	0.64 ± 0.24	*< 0.001*
Yes	81	4.15 ± 1.51		2.31 ± 1.23		0.98 ± 1.11		2.01 ± 1.43		115.59 ± 29.02		90.04 ± 28.80		0.80 ± 0.27	
Necrosis															
No	127	4.41 ± 1.36	0.183	2.38 ± 1.15	0.769	1.08 ± 0.33	*0.012*	2.24 ± 1.42	*0.010*	128.97 ± 26.07	*< 0.001*	83.57 ± 27.23	0.570	0.70 ± 0.26	*0.014*
Yes	32	4.04 ± 1.53		2.44 ± 1.16		0.92 ± 0.32		1.56 ± 0.81		104.53 ± 27.38		86.50 ± 27.49		0.83 ± 0.28	
ABI group															
> 1.3	0	-		-		-		-		-		-		-	
0.9 - 1.3	76	4.51 ± 1.25		2.44 ± 1.07		1.11 ± 0.30		2.25 ± 1.27		132.26 ± 25.38		81.25 ± 24.90		0.64 ± 0.25	
0.7 - 0.9	25	4.49 ± 1.78	0.196	2.35 ± 1.39	0.861	0.98 ± 0.35	0.118	2.86 ± 2.07	0.401	123.90 ± 31.39	*0.001*	90.75 ± 29.38	0.055	0.75 ± 0.23	*0.001*
0.4 - 0.7	44	3.95 ± 1.31		2.28 ± 1.14		1.01 ± 0.39		1.47 ± 0.64		120.38 ± 28.44		86.46 ± 28.64		0.80 ± 0.28	
< 0.4	14	4.29 ± 1.51		2.52 ± 1.22		0.92 ± 0.22		1.91 ± 0.85		116.48 ± 20.48		101.33 ± 26.46		0.89 ± 0.25	

Factor	n	TC (mmol/l)	p	LDLC (mmol/l)	p	HDLC (mmol/l)	p	TG (mmol/l)	p	ApoAI (mg/dL)	p	ApoB (mg/dL)	p	ApoB/A-I	p
		x̄ ± SD		x̄ ± SD		x̄ ± SD		x̄ ± SD		x̄ ± SD		x̄ ± SD		x̄ ± SD	
Rutherford (n = 85)															
II	2	3.65 ± 1.38		1.48 ± 1.58		1.24 ± 0.59		2.03 ± 1.73		150.40 ± 5.51		77.32 ± 13.81		0.51 ± 0.11	
III	25	4.27 ± 1.52		2.28 ± 1.27		1.03 ± 0.39		2.27 ± 1.92		124.53 ± 32.64		87.30 ± 31.25		0.80 ± 0.23	
IV	21	4.16 ± 1.52	0.976	2.22 ± 1.27	0.787	1.00 ± 0.36	0.446	2.26 ± 1.46	0.349	115.74 ± 20.71	*0.043*	92.67 ± 27.77	0.423	0.82 ± 0.27	*0.019*
V	28	4.07 ± 1.59		2.36 ± 1.14		0.97 ± 0.35		1.70 ± 0.96		109.29 ± 25.70		93.03 ± 25.49		0.88 ± 0.24	
VI	9	4.07 ± 1.37		2.64 ± 1.15		0.81 ± 0.14		1.37 ± 0.47		103.23 ± 35.07		94.06 ± 31.41		0.95 ± 0.38	
Stenosis exceeding 50%															
No	80	4.45 ± 1.28	0.288	2.42 ± 1.08	0.782	1.09 ± 0.31	0.114	2.23 ± 1.23	0.245	131.47 ± 24.61	*< 0.001*	81.05 ± 25.05	*0.022*	0.64 ± 0.24	*< 0.001*
Yes	79	4.22 ± 1.50		2.36 ± 1.21		1.00 ± 0.36		1.98 ± 1.46		116.55 ± 29.40		90.92 ± 28.57		0.81 ± 0.27	
Total occlusion															
No	114	4.51 ± 1.33	0.013	2.43 ± 1.11	0.477	1.08 ± 0.33	0.059	2.34 ± 1.46	*< 0.001*	128.93 ± 26.44	*< 0.001*	85.25 ± 26.13	0.608	0.68 ± 0.25	*0.009*
Yes	45	3.90 ± 1.46		2.29 ± 1.24		0.97 ± 0.33		1.50 ± 0.70		111.70 ± 28.44		87.72 ± 30.07		0.81 ± 0.28	

Factor	n	TC (mmol/l)		LDLC (mmol/l)		HDLC (mmol/l)		TG (mmol/l)		ApoAI (mg/dL)		ApoB (mg/dL)		ApoB/A-I	
		x̄ ± SD	**p**	**x̄ ± SD**	**p**	**x̄ ± SD**	**p**	**x̄ ± SD**	**p**	**x̄ ± SD**	**p**	**x̄ ± SD**	**p**	**x̄ ± SD**	**p**
Multi-level stenosis															
No	104	4.62 ± 1.34	< *0.001*	2.58 ± 1.14	*0.004*	1.08 ± 0.31	0.081	2.28 ± 1.43	*0.026*	130.05 ± 26.18	< *0.001*	86.48 ± 26.90	0.736	0.69 ± 0.25	*0.030*
Yes	55	3.78 ± 1.33		2.02 ± 1.08		0.98 ± 0.37		1.77 ± 1.11		112.73 ± 28.15		84.94 ± 28.05		0.78 ± 0.29	
PAD															
No	74	4.55 ± 1.25	0.065	2.47 ± 1.08	0.374	1.12 ± 0.30	0.013	2.25 ± 1.26	0.198	133.66 ± 24.16	< *0.001*	80.93 ± 25.10	*0.030*	0.63 ± 0.24	< *0.001*
Yes	85	4.14 ± 1.50		2.31 ± 1.20		0.98 ± 0.35		1.97 ± 1.42		115.69 ± 28.61		90.32 ± 28.38		0.81 ± 0.26	

PAD, peripheral artery disease; TC, total cholesterol; LDLC, low-density lipoprotein cholesterol; HDLC, high-density lipoprotein cholesterol; TG, triglycerides; ABI, ankle-brachial index; SD, standard deviation.

Variables in bold are statistically significant

We evaluated the discrimination between having PAD and not having PAD of the apoA-I, apoB and apoB/A-I ratio using the ROC curve (Figure 3). The AUROC was 0.714 (95%CI: 0.635 - 0.794); 0.300 (95%CI: 0.219 - 0.380) and 0.604 (95%CI: 0.516 - 0.692) for apoB/A-I ratio; apoAI and apoB, respectively. The probability apoB/A-I cutoff of 0.666 had a sensitivity of 0.682 and a specificity of 0.662.

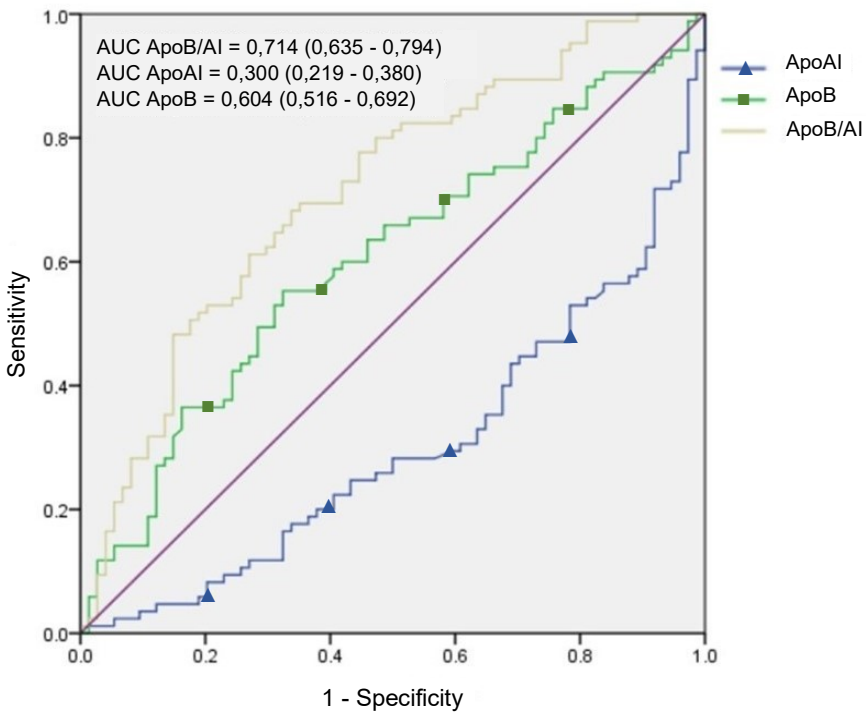

Chart 3. ROC curve of apoA-I, apoB and apoB/A-I ratio in the prediction of PAD in patients with diabetes

IV. DISCUSSION

Our results indicated that the apoB/A-I ratio demonstrated moderate discriminatory power in diagnosing PAD in diabetic patients. Additionally, there was an association between apolipoproteins and PAD clinical characteristics, as well as Doppler ultrasound features. Currently, there is no study assessing the association of apolipoproteins with peripheral artery stenosis, except for preliminary evidence from a systematic review comprising 22 studies, which indicated that, compared to the non-PAD group, the PAD group had higher levels of ApoB (MD: 12.5 mg/dL, 95%CI: 2.14 - 22.87), lower levels of ApoA-I (MD: -7.11 mg/dL; 95%CI: -11.94; -2.28), and higher ApoB/A-I ratio (MD: 0.11; 95%CI: 0 - 0.21).[10] However, recent study findings suggested that apoB/A-I ratio is associated with the severity of coronary artery stenosis, and paraoxonase (PON and cerebral artery stenosis.[11-14] Furthermore, evidence from recent studies indicated that apolipoprotein A-I and B are more effective than LDLC and other traditional lipid components in predicting the risk of occlusional events, including stroke and myocardial infarction.[4-8] These findings suggested that apolipoprotein A-I, B, and the apoB/A-I ratio could be associated with peripheral artery stenosis as well, making

them useful indicators for all occlusional events. Apolipoprotein B and A-I represent two aspects of the risk of atherosclerotic plaque formation: apoB reflects the pro-atherogenic characteristics, while apoA-I represents anti-atherogenic characteristics. Therefore, the apoB/apoA-I ratio simplifies the balance in the cholesterol transport process. A higher apoB/apoA-I ratio indicates a greater circulation of cholesterol in the blood, with an increased risk of cholesterol accumulation in arterial walls, leading to atherosclerotic plaque formation and occlusional events. Conversely, a lower apoB/apoA-I ratio signifies reduced cholesterol transport to the peripheral region, reinforcing reverse cholesterol transport and other beneficial functions, thereby reducing the risk of occlusional events.[5,6,15]

Our results indicated a limited correlation between traditional lipid profiles and the presence and characteristics of PAD in patients with diabetes. The primary reason explaining this difference was that the majority of patients in our study had been previously treated with statins. Therefore, plasma lipid indices at the time of examination do not accurately reflect the impact of pre-existing dyslipidemia. Recent studies have indicated that using traditional lipid parameters can lead to errors in assessing the risk of atherosclerosis. Post hoc analysis from large randomized controlled trials has shown that occlusional events occurred even in patients with LDL cholesterol at target levels.[16-18] One of the main reasons for inaccuracies in estimating the risk of heart disease through LDL cholesterol levels is the variation of this index between different laboratories and testing methods. In a meta-analysis based on cholesterol measurement trials (Cholesterol Treatment Trialist), the results revealed that the fluctuation in LDL cholesterol could be up to 0.5 mmol/L (20 mg/dL), leading to an estimated risk difference of up to 10% for occlusional events.[19] Additionally, LDLC testing does not accurately estimate LDL molecules representing the atherosclerotic process when disregarding the role of small, dense LDL molecules, which have been proven to exhibit higher atherogenic properties than larger molecules.

Our study has the following main limitations: First, patients in this study were all inpatients at the Vietnam National Heart Institute and Vietnam National Hospital of Endocrinology. Therefore, the patients, if not hospitalized for PAD, would typically be those with severe diabetic complications. As a result, the patient population in our study may not be representative of the general diabetic population. Second, there was a high prevalence of statin use among patients before study inclusion; however, adjustment for this factor was not feasible as the statin usage rate in the PAD group was 100%. We were also unable to adjust for the duration of statin use due to inaccuracies in medication recall and a considerable amount of missing data. Evidence from previous studies has shown that lipid-lowering medications, especially statins, directly impact apolipoproteins. Some medications have the potential to significantly reduce apoB levels, while others may increase apoA-I levels, or affect both types of apolipoproteins. Therefore, the use of statins may introduce biases in the association of the apolipoprotein with the primary outcomes of this study. Finally, the use of Doppler ultrasound instead of angiography to determine the study outcomes presents certain challenges. In diabetic patients, atherosclerotic lesions tend to progress peripherally, and the stenosis/occlusion are prone to occur in multiple arterial levels. This complexity poses difficulties in assessing stenotic degree using Doppler ultrasound, which inevitably leading to

inaccuracies in data collection.

V. CONCLUSION

Apolipoprotein AI and Apolipoprotein B are tests with considerable potential in diagnosing peripheral arterial disease in patients of type 2 diabetes mellitus patients. However, to ascertain the value of these tests, further studies are needed with larger sample sizes, on populations with a broader spectrum of diabetes and PAD severity, and employing imaging diagnostic modalities with higher reliability as the gold standard (MSCT angiography).

Acknowledgment statement

Not applicable for it.

Conflict of interests

The authors declare no competing interests in preparing this article.

Funding statement

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by and. The first draft of the manuscript was written by Ha Manh Nguyen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conceptualization: Nguyen Manh Ha and Dinh Thi Thu Huong; Methodology: Nguyen Manh Ha; Formal analysis and investigation: Nguyen Manh Ha, Nguyen Thi Ho Lan; Writing - original draft preparation: Nguyen Manh Ha; Writing - review and editing: Nguyen Manh Ha, Nguyen Thi Ho Lan, Pham Thai Binh, Vu Minh Phuc, Hoang Van Kien, Tran Thi Nhu Quynh, Nguyen Thi Nhu Quynh, Nguyen Thi Giang, Nguyen Thi Thu, Nguyen Thi Minh Thu, Nguyen Ngoc Mai, Nguyen Thi Ngoc Hoa, Dinh Thi Thu Huong; Resources: Nguyen Manh Ha; Supervision: Nguyen Manh Ha, Nguyen Thi Ho Lan and Dinh Thi Thu Huong.

Data availability statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

REFERENCES

1. Sun H, Saeedi P, Karuranga S, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract*. 2022;183:109119.

2. Almourani R, Chinnakotla B, Patel R, et al. Diabetes and Cardiovascular Disease: An Update. *Curr Diab Rep*. 2019;19(12):161.

3. American Diabetes Association. Peripheral arterial disease in people with diabetes. *Diabetes Care*. 2003;26(12):3333-3341.

4. Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med*. 2004;255(2):188-205.

5. Walldius G, Jungner I, Holme I, et al. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. *Lancet Lond Engl*. 2001;358(9298):2026-2033.

6. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet Lond Engl*. 2004;364(9438):937-952.

7. Walldius G, Aastveit AH, Jungner I. Stroke mortality and the apoB/apoA-I ratio: results of

the AMORIS prospective study. *J Intern Med*. 2006;259(3):259-266.

8. Bhatia M, Howard SC, Clark TG, et al. Apolipoproteins as predictors of ischaemic stroke in patients with a previous transient ischaemic attack. *Cerebrovasc Dis Basel Switz*. 2006;21(5-6):323-328.

9. Gerhard-Herman MD, Gornik HL, Barrett C, et al. 2016 AHA/ACC Guideline on the Management of Patients With Lower Extremity Peripheral Artery Disease: Executive Summary: A Report of the American College of Cardiology/ American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2017;135(12).

10. Forte F, Calcaterra I, Lupoli R, et al. Association of apolipoprotein levels with peripheral arterial disease: a meta-analysis of literature studies. *Eur J Prev Cardiol*. 2022;28(18):1980-1990.

11. Rahmani M, Raiszadeh F, Allahverdian S, et al. Coronary artery disease is associated with the ratio of apolipoprotein A-I/B and serum concentration of apolipoprotein B, but not with paraoxonase enzyme activity in Iranian subjects. *Atherosclerosis*. 2002;162(2):381-389.

12. Westerveld HT, van Lennep JE, van Lennep HW, et al. Apolipoprotein B and coronary artery disease in women: a cross-sectional study in women undergoing their first coronary angiography. *Arterioscler Thromb Vasc Biol*. 1998;18(7):1101-1107.

13. Hua R, Li Y, Li W, et al. Apolipoprotein B/A1 Ratio Is Associated with Severity of Coronary Artery Stenosis in CAD Patients but Not in Non-CAD Patients Undergoing Percutaneous Coronary Intervention. *Dis Markers*. 2021;2021:8959019.

14. Li MM, Lin YY, Huang YH, et al. Association of Apolipoprotein A1, B with Stenosis of Intracranial and Extracranial Arteries in Patients with Cerebral Infarction. *Clin Lab*. 2015;61(11):1727-1735.

15. Walldius G, Jungner I, Aastveit AH, et al. The apoB/apoA-I ratio is better than the cholesterol ratios to estimate the balance between plasma proatherogenic and antiatherogenic lipoproteins and to predict coronary risk. *Clin Chem Lab Med*. 2004;42(12):1355-1363.

16. Zhan S, Tang M, Liu F, et al. Ezetimibe for the prevention of cardiovascular disease and all-cause mortality events. *Cochrane Database Syst Rev*. 2018;11(11):CD012502.

17. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. *N Engl J Med*. 2018;379(22):2097-2107.

18. Giugliano RP, Pedersen TR, Park JG, et al. Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a prespecified secondary analysis of the FOURIER trial. *Lancet Lond Engl*. 2017;390(10106):1962-1971.

19. Cholesterol Treatment Trialists' (CTT) Collaborators, Mihaylova B, Emberson J, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet Lond Engl*. 2012;380(9841):581-590.