



Asymptomatic Plaques of Lower Peripheral Arteries and Their Association with Cardiovascular Disease: An Autopsy Study

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Aim: Patients with peripheral artery disease (PAD) have a high prevalence of cardiovascular morbidity and mortality; however, majority of patients with PAD are asymptomatic. This study aimed to histologically evaluate whether asymptomatic, lower extremity artery plaques are associated with systemic atherosclerosis and the onset of cardiovascular disease (CVD) events using autopsy cases.

Methods: We histologically investigated the atherosclerotic plaques of the common iliac, common carotid, coronary, and renal arteries from 121 autopsy cases without symptoms of PAD (mean age: 67.6 years; 63% men; 83% non-CVD death). We evaluated the relationship between the degree of iliac artery atherosclerosis and that of other arteries, and also the presence of any CVD, myocardial infarction, stroke, and renal failure.

Results: Advanced atherosclerotic plaques (American Heart Association ≥ 4) were present in 86 (72%) common iliac arteries in these cases. These arteries also showed high frequencies of calcification (66%), intraplaque hemorrhage (42%), and plaque disruption (24%). These advanced lesions were associated with age (≥ 60 years), sex (male), hypertension, diabetes, and smoking habit (all $P < 0.05$). Additionally, it was significantly associated with CVD (odds ratio, 95% confidence interval; 6.2, 2.2–22), myocardial infarction (6.4, 1.2–19), stroke (8.7, 1.7–16), and renal failure/hemodialysis (5.8, 1.1–11). Cases with advanced iliac artery plaques had advanced coronary and carotid atherosclerosis.

Conclusion: These results indicate that asymptomatic advanced plaques are frequently observed in common iliac arteries, and are associated with generalized atherosclerosis and CVD events.

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Key words: Asymptomatic peripheral artery disease, Generalized atherosclerosis, Cardiovascular disease, Autopsy

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Introduction

Peripheral arterial disease (PAD) is most commonly caused by atherosclerosis. Risk factors for development of PAD include smoking, diabetes, hyperten-

sion, and hypercholesterolemia¹. An advanced stage of PAD shows symptoms, including intermittent claudication, foot pain at rest, and/or tissue necrosis. This disease affects more than 200 million people worldwide and is a common cause of vascular morbidity². Therefore, the economic impact of PAD is significant^{3,4}. Patients with PAD have a high prevalence of cardiovascular morbidity and mortality¹. Therefore, diagnosis and treatment of PAD are important; however, the majority of patients with PAD are asymptomatic⁵⁻⁸. The incidence of asymptomatic PAD and whether it is associated with cardiovascular disease

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(CVD) events are still unclear.

Aim

This study aimed to investigate the common iliac arteries of autopsy cases with no symptoms of PAD and to evaluate whether the presence of asymptomatic iliac artery atherosclerosis was associated with carotid, coronary, and renal atherosclerosis, and also the onset of CVD events.

Methods

Study Population and Design

One hundred twenty-one autopsy cases (mean age, 67.6 ± 12.6 years; 63% male) (**Table 1**) were studied, and were autopsied at University of Miyazaki Hospital, Faculty of Medicine, University of Miyazaki, Japan, from 2000 to 2005. Autopsy cases with a history of intermittent claudication, rest pain, limb amputation, or previous intervention, including angioplasty of the lower legs, were excluded from this study. The causes of death were as follows: CVD (i.e., coronary artery disease, stroke, and abdominal aortic rupture, $n=21$ [17%]); malignancy ($n=55$, 46%); infection or sepsis ($n=22$, 18%); and other causes (liver cirrhosis, collagen disease, amyotrophic lateral sclerosis, or intestinal pneumonia, $n=23$ [18%]). Hypertension was defined as blood pressure $\geq 140/90$ mmHg or the use of an antihypertensive agent. Diabetes mellitus was defined as follows: a fasting glucose level ≥ 126 mg/dL; a random nonfasting glucose level ≥ 200 mg/dL; hemoglobin A1c level $\geq 6.5\%$; or the use of an antihyperglycemic agent. Hyperlipidemia was defined as follows: a total cholesterol level ≥ 220 mg/dL; low density lipoprotein level ≥ 140 mg/dL; triglyceride level ≥ 150 mg/dL; or the use of an oral lipid-lowering agent. Renal failure was defined as a GFR < 15 mL/min per 1.73 m² or treatment requiring dialysis. The Ethical Committee of the University of Miyazaki approved the study protocol (No. 2015-186). The study was performed in accordance with the ethical standards of the Declaration of Helsinki.

Histological Examination

The following vascular structures and organs were isolated from 10% formalin-fixed organs, as described elsewhere^{9, 10}: bilateral common iliac arteries; bilateral common carotid arteries; bilateral renal arteries, and the coronary artery (main left coronary artery). We cut the arteries longitudinally for macroscopic observation, and all arteries were immersion-fixed. Isolated arteries were cut transversely at 3 mm, and segments of vessels showing the most stenosis were selected for

Table 1. Characteristics of the autopsy subjects ($n=121$)

Age, years	67.6 \pm 12.6
Male, n (%)	76 (63)
Diabetes, n (%)	27 (22)
Hypertension, n (%)	51 (42)
Hyperlipidemia, n (%)	9 (7)
Statin use, n (%)	4 (3.3)
Smoking, n (%)	61 (50)
Cause of death	
Malignancy, n (%)	55 (46)
Cardiovascular disease, n (%)	21 (17)
Infection or sepsis, n (%)	22 (18)
Others, n (%)	23 (18)

Values are n (%) or mean \pm SD.

histological examination.

Two pathologists (E.N. and Y.S.) who were unaware of the patients' characteristics performed histological examination of the arteries. Histological sections were paraffin-embedded and stained with hematoxylin and eosin. We assessed advanced atherosclerotic lesions in the systemic arteries and classified six types of atherosclerotic lesions in accordance with the definition proposed by the Committee on Vascular Lesions of the Council on Atherosclerosis, American Heart Association (AHA)⁹. On the basis of the classification system defined by the AHA classification, advanced lesions included the necrotic core (Type IV), fibroatheroma (Type V), and plaque rupture or thrombus formation (Type VI) (**Fig. 1**). To assess the severity of atherosclerosis in the vasculature, we also evaluated calcification, intraplaque hemorrhage, and plaque disruption, and measured the lipid area of the plaque (%) and the maximum intima/media layer ratio (I/M ratio). The lipid area, as ratio (%) of total area of intima, and intima and media thickness were measured using an image analysis system (Win Roof, Mitani, Fukui, Japan). All arteries were measured under nonperfusion-fixed conditions.

Statistical Analysis

All statistical analyzes were performed with JMP version 8.0.1 (SAS, Cary, NC) and GraphPad Prism 5.01 (GraphPad Software, San Diego, CA). Variables with a normal distribution are expressed as means \pm standard deviation (SD), whereas variables with a skewed distribution are expressed as median with interquartile range. The associations between individual variables were calculated by Spearman's correlation method using raw data. The clinical parameters of the patients with or without risk factors were compared using the unpaired *t*-test for variables with a normal

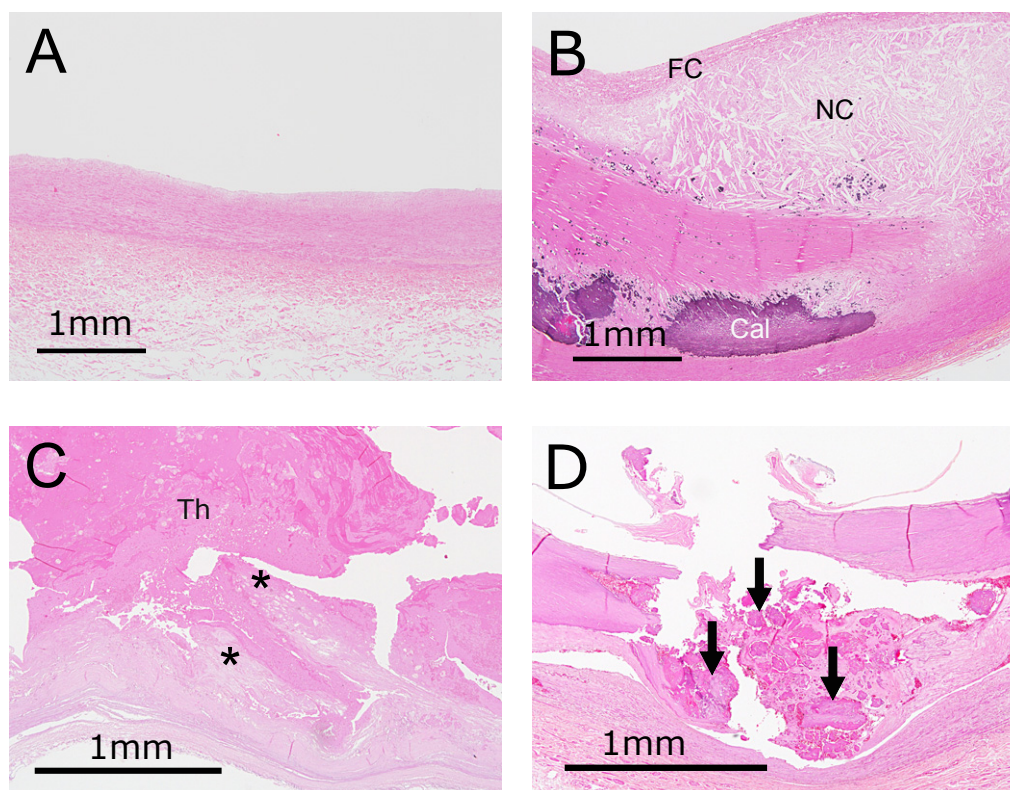


Fig. 1. Histological findings of the iliac arteries.

A. Normal artery without atherosclerotic plaques.

B. Advanced plaque. A necrotic core contains a large number of cholesterol crystals, a thin fibrous cap (< 65 μm), and calcification. NC; necrotic core, Cal; calcification, FC; fibrous cap.

C. Plaque rupture (*) with thrombus. Th; thrombus.

D. Calcified nodules. Irregular blocks of calcific nodules (arrows) are surrounded by hemorrhage and fibrin, associated with a disrupted fibrous cap.

distribution or the Mann–Whitney test for variables with a skewed distribution. Categorical parameters were compared with the chi-square test or Kruskal–Wallis test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression analysis. A P value of <0.05 was considered statistically significant.

Results

Advanced atherosclerotic plaques (AHA ≥ 4) in the iliac arteries were observed in 86 (72%) subjects (**Table 2**). These lesions showed frequent calcification (66%) and intraplaque hemorrhage (42%). Plaque disruption was found in 29 subjects (plaque rupture, 19%; plaque erosion, 5%). The lesions showed a high I/M ratio (7.5 ± 6.9) and large lipid area ($19\% \pm 20\%$) (**Fig. 1**).

The presence of asymptomatic advanced plaques in the iliac arteries was strongly associated with aging (≥ 60 years), hypertension, and diabetes. Asymptom-

Table 2. Histological characteristics of iliac artery plaques

Plaque features	
AHA advanced lesion	86 (72)
Calcification	80 (66)
Intraplaque hemorrhage	52 (42)
Any plaque disruption	29 (24)
Any plaque rupture	23 (19)
Rupture without thrombus	11 (9)
Rupture with thrombus	12 (10)
Erosion with thrombus	6 (5)
Lipid area (%)	19 ± 20
I/M ratio	7.5 ± 6.9

Values are n (%) or mean \pm SD.

atic advanced plaques were also frequency increased with sex (male) and smoking habit, but not with hyperlipidemia (**Table 3**). Multilogistic analysis showed that aging and hypertension were independently associated with advanced plaques. The degree of atherosclerosis

Table 3. Odds ratios for clinical risk factors according to advanced lesions (AHA ≥ 4) of the iliac artery

	Uni-logistic regression	Multi-logistic regression
≥ 60 years	6.2 (2.52–15.68)***	5.7 (2.05–12.18)**
Sex (male)	2.9 (1.29–6.72)**	n.s.
Hypertension	8.6 (3.00–30.91)***	4.3 (1.32–16.93)*
Diabetes mellitus	6.5 (1.79–42.36)**	n.s.
Hyperlipidemia	n.s.	–
Smoking habit	2.8 (1.22–6.58)*	n.s.

Values indicate odds ratios (95% confidence intervals). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.s., not significant

(I/M ratio and prevalence of AHA ≥ 4) increased in an age-related manner in both sexes (**Fig. 2**). For subjects with age in their 50s and 60s, the degree of atherosclerosis was higher in men than in women, but this difference was not significant.

We then examined the correlations between advanced iliac plaques and other arteries (coronary, carotid, and renal arteries). Advanced plaques in the coronary or carotid arteries were frequently observed in subjects with asymptomatic iliac plaques (**Table 4**). Additionally, multiple advanced plaques were present in 64 (75%) subjects with iliac asymptomatic plaques.

Finally, we examined the relationship between iliac asymptomatic plaques and clinical findings (any CVD, myocardial infarction, stroke, or renal failure/hemodialysis). The presence of asymptomatic iliac plaques was strongly associated with any CVD (OR, 95% CI; 6.2, 2.2–22), myocardial infarction (6.4, 1.2–19), stroke (8.7, 1.7–16), and renal failure or hemodialysis (5.8, 1.1–11) (**Table 5**).

Discussion

In this autopsy study, we found that the advanced iliac artery plaques were frequently present in patients without symptoms of PAD, and were associated with systemic atherosclerosis and CVD events.

A recent study showed that the subclinical atherosclerotic plaques were present in 63% of middle-aged participants, and plaques were most common in iliac–femoral arteries¹¹. In a population-based point-prevalence study, Sigvant *et al.*¹² examined 5080 patients with PAD and showed that only one-third of patients were symptomatic. The women's Health and Aging Study also reported that 63% of women with PAD were asymptomatic¹³. Takahara *et al.*¹⁴ analyzed a database of 559 Japanese patients with critical limb ischemia who participated in a multicenter, prospective study. They showed that the prevalence of the absence of prior intermittent claudication was 50% in patients with critical limb ischemia. This evidence

indicates that the majority of patients with PAD are asymptomatic. Collins *et al.*¹⁵ reported that women remain asymptomatic for longer periods of time and present later in life with more advanced disease. Our study showed advanced atherosclerotic lesions in iliac arteries in nearly 70% of participants who had no symptoms of PAD. These findings suggest that asymptomatic advanced atherosclerosis is common in middle-aged and older people.

Comparative pathological studies of atherosclerosis in different arteries have been reported, but many of these studies were performed on gross specimens. Histological studies on atherosclerosis have been recently reported. Dalager *et al.*¹⁶ examined 100 autopsy cases (20–82 years old) and found that femoral atherosclerosis was less advanced, and severe atherosclerotic lesions were dominated by fibrous plaques. They also reported that superficial femoral artery atherosclerosis was significantly associated with coronary death¹⁷; however, Vink *et al.*¹⁸ examined atherosclerotic femoral arteries from 42 individuals and frequently observed large, lipid-rich plaques. Pasterkamp *et al.*¹⁹ reported that plaque burden of the common carotid artery was weakly correlated with that of other peripheral arteries (iliac, femoral, and renal arteries) in an autopsy study. Nevertheless, they did not examine the association between the coronary artery and peripheral arteries. The present study showed that asymptomatic iliac advanced lesions were correlated with carotid, coronary, and renal arteries, and also with any CVD events. This evidence suggests that atherosclerosis in the lower extremities with or without symptoms of PAD may be correlated with systemic atherosclerosis and CVD events.

Development of PAD is associated with age, hypertension, diabetes, cholesterol, and smoking¹. We showed that advanced age, sex, hypertension, diabetes, and smoking habit were risk factors for histological iliac artery atherosclerosis. Epidemiological studies have shown that the onset of CVD, a major manifestation of atherosclerosis, occurs at an average of 10 years later

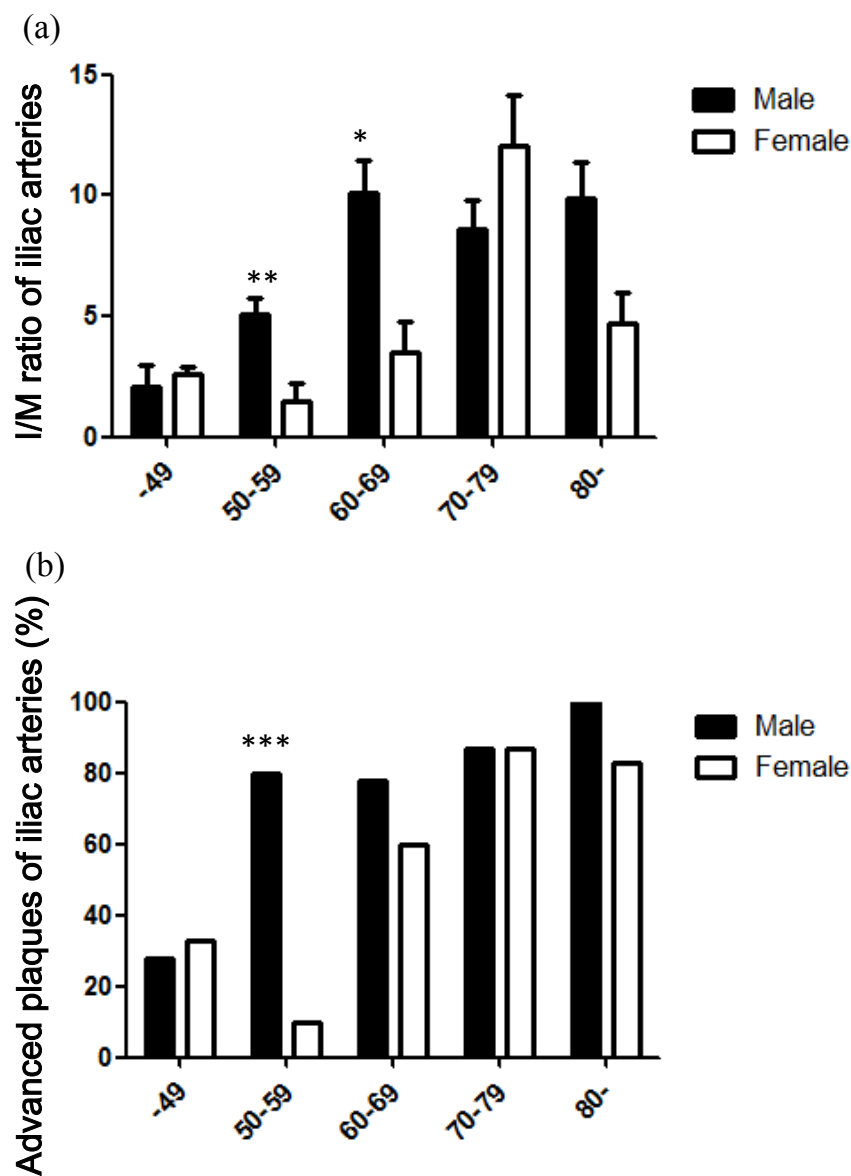


Fig. 2. Sex-specific I/M ratio (a) and prevalence of advanced plaques (AHA ≥ 4) (b) of the iliac arteries by age groups (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table 4. Relationships between advanced lesions (AHA ≥ 4) of the iliac artery and advanced lesions of other arteries

	Iliac non-advanced lesion ($n = 35$)	Iliac advanced lesion ($n = 86$)	P value
Coronary plaque	6 (18%)	51 (59%)	<0.0001
Carotid plaque	1 (3%)	45 (52%)	<0.0001
Renal plaque	1 (3%)	14 (16%)	0.0642
Any multiple plaques	1 (3%)	64 (75%)	<0.0001
Two plaques	1 (3%)	28 (33%)	0.0001
Three plaques	0 (0%)	31 (37%)	<0.0001
Four plaques	0 (0%)	5 (6%)	0.3203

Fisher's exact test was used to determine the relationships between advanced lesions of the iliac artery and advanced lesions from other arteries.

Table 5. Odds ratios for advanced plaques (classified by the AHA) of the iliac artery and clinical findings

	Odds ratio	<i>P</i> value
Cardiovascular disease	6.2 (2.21–22.3)	0.0002
Myocardial infarction	6.4 (1.21–18.6)	0.0255
Stroke	8.7 (1.69–16.4)	0.0059
Renal failure or hemodialysis	5.8 (1.11–10.8)	0.0364

The values indicate odds ratios (95% confidence intervals).

in women than in men, with myocardial infarction occurring 20 years later^{13, 20}); however, a sex- and age-specific prevalence of PAD has not been clearly shown. We showed that atherosclerosis was more severe in men than in women in their 50s and 60s, but this difference was not observed in their 70s (**Fig. 2**). Sawabe *et al.*²¹) also reported similar findings in an autopsy study. Therefore, menopause plays a decisive role in the increase of CVD risk in women. Estrogen decreases oxidative stress, induces vasodilation, and inhibits vascular smooth muscle proliferation²²).

Intima-media thickness (IMT) is an established index for evaluation of atherosclerosis. In particular, ultrasound-determined carotid IMT is widely used as an indicator of generalized atherosclerotic burden and a predictor of CVD events^{23–25}). We previously performed an autopsy analysis to examine the associations of microscopy-determined carotid IMT with the severity of atherosclerosis in the generalized arteries²⁶). We found that advanced iliac atherosclerosis increased in accordance with increasing microscopy-determined carotid IMT²⁶). Iwamoto *et al.*²⁷) recently evaluated IMT of the leg arteries and showed that IMT was significantly larger in the atherosclerotic PAD group than in the control group. In our study, the I/M ratio of the iliac arteries was associated with that of the coronary and carotid arteries, but not with that of the renal arteries (data not shown).

There are several limitations in our study. First, the group size, especially young people and women, was small. Second, because the source for risk factors was limited to a single postmortem collection, the duration of risk factor exposure or treatment was not evaluated.

Conclusion

Our study shows that asymptomatic plaques in arteries of the lower extremity are frequently observed and associated with generalized atherosclerosis and CVD events. These findings suggest that the detection of asymptomatic PAD is a predictive marker of systemic atherosclerotic disease or CVD.

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Conflicts of Interest

Sato Y. receives Grans-in-Aid for Scientific Research in Japan (No. 26460437). Nakamura E, Iwakiri T, Yamashita A, Moriguchi-Goto S, Maekawa K, Gi T, and Asada Y. have no conflict interest.

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