≥ *Original Article* ≤

Infrainguinal Lesion of Peripheral Artery Disease and Levels of ω-3 Polyunsaturated Fatty Acids in Peripheral Artery Disease

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Background: Recently, reduced serum levels of ω-3 polyunsaturated fatty acids (PUFAs) including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been focused upon as newly recognized risk factors for peripheral artery disease (PAD). The present study investigated the association between disease location and serum levels of ω-3 PUFAs in patients with PAD.

Methods: We retrospectively analyzed the data of patients at Tokyo Medical University between August 2011 and November 2015. The subjects included 98 patients who were categorized into two groups: those with (n=72) and without infrainguinal lesions (n=26).

Results: Univariate analysis revealed that low ankle–brachial pressure index (ABI) values, low EPA levels, low DHA levels, low triglyceride levels, and diabetes mellitus were significant risk factors for infrainguinal lesions. Multivariate analysis indicated that low ABI values [p=0.018; odds ratio, 0.043; 95% confidence interval (CI), 0.003–0.579] and low DHA levels (p=0.003; odds ratio, 0.986; 95%CI, 0.977–0.995) were significant independent risk factors for infrainguinal lesions.

Conclusion: Our study demonstrated that reduced serum level of DHA may underlie the presence of infrainguinal lesions in patients with PAD.

Introduction

The overall incidence of peripheral artery disease (PAD) is 2.7%–7.9% in Japan, and the number of patients has been increasing in the high-age society.[1] PAD also tends to occur more frequently in conjunction with diabetes mellitus (DM) and dyslipidemia. To date, various risk factors for symptomatic or asymptomatic PAD have been reported, including male sex, aging, DM, smoking, hypertension, dyslipidemia, hyperhomocysteinemia, Asian/Hispanic/Black races, C-reactive protein (CRP) level, and renal insufficiency.[2] Aging is the most important non-modifiable risk factor and increases the risk of PAD by 2- to 3-fold, whereas the most important modifiable risk factors include smoking and DM, which increase the risk of PAD by 3- to 4-fold.

The locations of PAD vary as follows: femoropopliteal artery in 50%, aortoiliac artery in 24%, tibiofibular artery in 17%, and popliteal artery in 5% of patients.[3] These variations are divided roughly into three major patterns of arterial obstruction: proximal lesions (suprainguinal lesions), distal lesions (infrainguinal lesions), and a combination of lesions.[4,5] Patients with proximal lesions more commonly present with intermittent claudication, whereas those with distal lesions frequently have limb-threatening ischemia. Furthermore, several studies have suggested that the etiology of the disease process in the lower extremities may vary depending on the anatomic site of the lesion.[6] Different clinical risk factors, especially atherosclerotic risk factors, predict the involvement of different arterial segments.

Recently, the reduced levels of plasma ω-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have been established as newly recognized risk factors for PAD.[7–10] ω-3 PUFAs—found in fish oils—are known to decrease

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the production of inflammatory eicosanoids, cytokines, and reactive oxygen species and reduce the expression of adhesion molecules.[11)] Therefore, ω-3 PUFAs have various beneficial effects in lowering the blood pressure and heart rate, improving dyslipidemia, reducing inflammation, and improving vascular and platelet function. Although numerous studies have assessed the role of ω-3 PUFAs in patients with PAD,[7–10)] none have investigated the association of ω-3 PUFAs with the disease location in PAD.

We therefore hypothesized that ω-3 PUFAs, especially EPA and DHA, are associated with disease location in PAD and investigated the association between the disease location and coexisting risk factors, including ω-3 and ω-6 PUFAs, in patients with PAD.

Patients and Methods

Patients
This study was approved by the institutional ethics committee of Tokyo Medical University and performed from August 2011 to November 2015, in accordance with the Declaration of Helsinki (2001). The subjects included 98 consecutive patients with PAD who underwent or were scheduled to undergo endovascular therapy (EVT) and/or bypass surgery at Tokyo Medical University Hospital; imaging studies were performed to identify the disease location only in these patients. Exclusion criteria involved patients who denied written informed consent, patients who had undergone previous revascularization for PAD, and patients who were being treated with oral ω-3 PUFA.

The disease location was evaluated using three-dimensional computed tomography angiography, digital subtraction angiography, or duplex scanning. These patients were categorized into two groups: 72 patients (73.5%) with infrainguinal lesions (Group A) and 26 patients (26.5%) without infrainguinal lesions (Group B).

Baseline analysis
The study participants completed questionnaires regarding their health status, medical history, and cardiovascular risk factors and underwent clinical examination. Clinical predictors, including age, male sex, body mass index (BMI), critical limb ischemia (CLI), smoking history, current smoking habits, hypertension, dyslipidemia, DM, history of cerebrovascular disease (CVD), history of ischemic heart disease (IHD), and the presence of end-stage renal failure managed with hemodialysis (HD), were included in the analyses. CLI was defined as Fontaine stages III (pain at rest) and IV (ulcers and gangrene). Hypertension was defined as a systolic/diastolic blood pressure of 140/90 mmHg or if a patient was administered hypertensive medication. DM was diagnosed if a patient had hemoglobin A_{1C} level of 6.5%, fasting plasma glucose

concentration of 126 mg/dL, history of taking any antihyperglycemic medications, or previous diagnosis of diabetes. The ankle–brachial pressure index (ABI) was evaluated using the ABI Form (Omron-Colin, Tokyo, Japan) at our vascular laboratory.

Measurement of serum lipids
To assess lipid profiles, blood samples were obtained from peripheral veins. Serum levels of ω-3 PUFAs, including EPA, DHA, arachidonic acid (AA), and dihomo-γ-linolenic acid (DHLA), were measured at SRL Inc., Tokyo, Japan, using a GC-2010 gas chromatograph (Shimadzu Corporation, Kyoto, Japan) and a TC-70 column (GL Sciences B.V., Tokyo, Japan). Serum levels of lipids, including total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL), and low-density lipoprotein (LDL), were assessed using standardized enzymatic methods at our hospital.

Statistical analysis
All statistical analyses were performed using SPSS 22 (IBM Corporation, Armonk, New York, USA). Continuous values are expressed as mean ± standard deviation, and categorical data are expressed as numbers (n) and percentages in parentheses. For continuous data, the normality of the distribution was examined using the Kolmogorov–Smirnov test. Univariate analysis was performed using the Student t-test for normally distributed data and Mann–Whitney U test or chi-square test for other data. Statistically significant variables in univariate analyses were entered into the multivariate model to assess their independent impact on the disease location. In selecting variables, the forward selection method was applied according to the likelihood ratio. The Hosmer–Lemeshow test was used to determine how well the multiple logistic regression model fit our data.

Results

Clinical characteristics of the subjects
The patient characteristics are shown in **Table 1**. Notably, the serum levels of ω-3 PUFAs also are shown in **Table 1**: DHA, 139.2 ± 53.3 μg/mL; EPA, 70.5 ± 41.4 μg/mL; AA, 174.2 ± 44.8 μg/mL; and DHLA, 36.0 ± 13.8 μg/mL.

Univariate analyses concerning disease locations
Table 2 shows comparisons of the clinical characteristics and serum lipid levels between Groups A and B. There were no differences in the values or incidences of age, male sex, BMI, smoking, hypertension, CVD, IHD, and HD, whereas the incidence of DM in Group A was significantly higher than that in Group B (p = 0.046). The value of ABI was significantly lower in Group A than in Group

Table 1 Patient characteristics

Variable	
Number of patients	98
Age, years	73±7.3
Male sex	79 (81%)
Infrainguinal lesion	72 (73.5%)
CLI	26 (26.5%)
ABI	0.56±0.21
BMI, kg/m²	22.4±3.3
Smoking	72 (73.5%)
Comorbidities	
Hypertension	72 (73.5%)
DM	43 (43.9%)
CVD	31 (31.6%)
IHD	36 (36.7%)
HD	13 (13.3%)
ω-3 fatty acids	
EPA, μg/mL	70.5±41.4
DHA, μg/mL	139.2±53.3
AA, μg/mL	174.2±44.8
DHLA, μg/mL	36.0±13.8
Serum lipids	
TC, mg/dL	176.4±36.2
TG, mg/dL	151.7±85.9
HDL, mg/dL	46.36±13.9
LDL, mg/dL	99.81±33.5

The values are presented as n (%) or mean±standard deviation, as appropriate. CLI: critical limb ischemia; ABI: ankle–brachial pressure index; BMI: body mass index; DM: diabetes mellitus; CVD: cerebrovascular disease; IHD: ischemic heart disease; HD: hemodialysis; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; DHLA: dihomo-γ-linolenic acid; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein

Table 2 Univariate analyses concerning disease location

Variable	Group A (n=72)	Group B (n=26)	p value
Age, years	73.4±7.3	72.0±7.4	0.398
Male sex, %	79.2	84.6	0.547
ABI	0.54±0.21	0.64±0.20	0.046*
BMI, kg/m²	22.0±3.4	23.4±2.9	0.072
Smoking, %	75.0	69.2	0.568
Comorbidities			
Hypertension, %	72.2	76.9	0.642
DM, %	50.0	26.9	0.046*
CVD, %	34.7	23.1	0.274
IHD, %	36.1	38.5	0.831
HD, %	16.7	3.8	0.099
Serum ω-3 fatty acids			
EPA, μg/mL	65.1±38.6	85.3±46.6	0.038*
DHA, μg/mL	130.1±47.6	164.4±61.8	0.008*
AA, μg/mL	173.2±46.1	176.8±42.7	0.733
DHLA, μg/mL	35.8±14.1	36.5±13.7	0.832
Serum lipids			
TC, mg/dL	175.0±37.6	180.2±33.2	0.531
TG, mg/dL	140.8±84.4	182.0±86.1	0.048*
HDL, mg/dL	46.9±13.6	44.9±15.2	0.523
LDL, mg/dL	99.7±35.8	100.0±27.5	0.968

* p value significant. Abbreviations as in **Table 1**.

Table 3 Multivariate analyses concerning disease location

Variable	p value	Odds ratio	95%CI
ABI	0.018*	0.043*¹	0.003–0.579
DHA	0.003*	0.986*¹	0.977–0.995

* p value significant. *¹ Ratio when a variable changes by 1. Abbreviations as in **Table 1**.

B (p=0.046). The serum levels of EPA, DHA, and TG in Group A were significantly lower than those in Group B (p=0.038, 0.008, and 0.048, respectively), but there were no differences in the serum levels of DHLA, AA, TC, HDL, and LDL.

Multivariate analyses concerning disease locations

Multiple regression analysis using significant factors detected in the univariate analyses was performed to identify determinants of the presence of infrainguinal lesions. Consequently, ABI values [p=0.018; odds ratio, 0.043; 95% confidence interval (CI), 0.003–0.579] and DHA levels (p=0.003; odds ratio, 0.986; 95%CI, 0.977–0.995) were found to be significant factors associated with the presence of infrainguinal lesions (**Table 3**). In contrast, age, male sex, DM, and TG levels did not show any significant correlations with the presence of infrainguinal lesions. The p value for Hosmer–Lemeshow statistics was 0.085

(>0.05), implying that our logistic model fit the data well.

Discussion

The reasons why atherosclerosis occurs at certain sites, such as the aortoiliac, femoropopliteal, and orbital arteries, remain unclear. Hypotheses explaining the site selectivity of atherosclerotic lesions include hemodynamic stress related to arterial geometry and anatomic, cellular, or biochemical variations in the arterial wall.[6,12)] Chen et al. enumerated several specific factors such as the iliac arteries are characterized by elastic properties, whereas the femoropopliteal and tibial arteries contain progressively more muscular elements[6,13)]; furthermore, the arterial lumen-to-wall thickness decreases from proximal to distal segments, generating both an alteration in arterial flow and shear stress associated with atherosclerosis.[6,14)] Therefore, the involvement of different arterial segments

may be affected by different clinical factors.

Several studies have demonstrated that the distribution and extent of PAD are affected by atherosclerotic risk factors.[6] Most recently, Diehm et al. analyzed a consecutive series of 2,659 patients with PAD undergoing EVT and reported that smoking is closely related to proximal disease, whereas higher age and DM are predisposing factors to the involvement of distal disease.[14] Moreover, a striking difference is evident, with more femoropopliteal target lesions detected in female patients and more iliac and infragenicular target lesions in male patients.

ω-3 PUFAs have been focused upon as new atherosclerotic risk factors. It has been reported that reduced plasma levels of ω-3 PUFAs are involved in the deterioration observed in IHD. Low serum EPA levels and low serum EPA to serum AA (EPA/AA) ratios are associated with high vulnerability for coronary plaques.[15] Atherosclerotic plaques readily incorporate EPA, and a higher plaque EPA content is associated with decreased numbers of foam cells and T cells, less inflammation, and increased stability.[16] Until recently, these favorable effects have been primarily attributed to EPA, which is present in large amounts in fish oil. However, several studies now demonstrate that DHA, although often present in lower quantities, has equally important anti-arrhythmic, anti-thrombotic, and anti-atherogenic effects.[17]

Several clinical studies have indicated a significant association between serum levels of EPA or ratios of EPA/AA and the incidence of major adverse cardiac events after patients with acute myocardial ischemia underwent percutaneous coronary intervention.[18–20] Although little has been reported regarding DHA, higher plasma DHA levels are associated with the reduced progression of coronary atherosclerosis in women with IHD.[21] Moreover, the intake of EPA and/or DHA in fish oil has been proven to attenuate the risk of CVD, IHD, and sudden cardiac death in patients with hypercholesterolemia, peripheral artery disease, or atrial fibrillation.[22–24]

More recently, decreased plasma levels of ω-3 PUFAs, including EPA and DHA, have been established as newly recognized factors for PAD. Fujihara et al. demonstrated that reduced plasma EPA/AA ratios may underlie PAD in Japanese patients. ABI is an independently associated factor for PAD.[7] Fukuda et al. reported that patients with PAD were more likely to have a low EPA/AA ratio, and non-diabetic patients with PAD had a significantly reduced EPA/AA ratio.[8] Sugiura et al. reported that significant relationships among EPA, CRP, and PAD were confirmed in patients with coronary artery disease (CAD). High CRP levels and low EPA levels were significant and independent predictors of PAD.[9] Leng et al. revealed that when subjects with PAD were compared with healthy control subjects, the largest differences were noted in plasma levels of ω-3 PUFAs, particularly DHA.[10]

This study demonstrates that statistically significant associations exist between serum levels of DHA (but not EPA) and the patterns of disease location in patients with PAD. DHA (p = 0.003; odds ratio, 0.986; 95%CI, 0.977–0.995) was found to be a significant risk factor for the presence of infrainguinal lesions. This result may be explained by the differing hemodynamic and anti-atherogenic properties of EPA and DHA.[17] DHA is more effective than EPA in reducing blood pressure, and these blood pressure-lowering effects correlate with improvements in endothelial relaxation and attenuated vascular constriction.[25] Platelet aggregatory responses ex vivo and platelet-derived thromboxane A_2 production are reduced by DHA and not EPA.[26] Therefore, reduced serum DHA levels may result in greater vascular constriction and platelet aggregation, leading to progression to smaller arteries, such as the infrainguinal arteries.

In this study, ABI was also identified as a significant factor associated with the presence of infrainguinal lesions, and this finding is in agreement with a previous report; ABI values were slightly higher in aortoiliac lesions than in femoropopliteal lesions.[27]

This study has several limitations. First, the data were retrospectively analyzed from a prospectively maintained database of only patients undergoing EVT. Therefore, there may be some bias in relation to the selection of patients. Second, the cross-sectional nature of the study design may not have allowed the evaluation of the direct effect of DHA on the progression of PAD. Third, the drugs and supplements were assessed but dietary habits were not assessed in this study. This may have affected the outcome of the study.

Conclusion

This study demonstrates that decreased serum levels of DHA and not EPA may underlie the presence of infrainguinal lesions in patients with PAD. Further prospective cohort studies are required to confirm this result.

Disclosure Statement

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

Author Contributions

Conception and design: TN, TI, HO
Analysis and interpretation: TN, TI, HO
Data collection: TN, TI
Writing the article: TN, TI

Critical revision of the article: TN, TI, HO
Statistical analysis: YO, SI
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References

1) Fujiwara T, Saitoh S, Takagi S, et al. Prevalence of asymptomatic arteriosclerosis obliterans and its relationship with risk factors in inhabitants of rural communities in Japan: Tanno-Sobetsu study. Atherosclerosis 2004; **177**: 83-8.

2) Norgren L, Hiatt WR, Dormandy JA, et al. Inter-society consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg 2007; **45 Suppl S**: S5-67.

3) Veith FJ, Haimovici H. Femoropopliteal arteriosclerotic occlusive disease. In: Haimovici H, Ascer E, Hollier LH, et al. eds. Haimovici's Vascular Surgery, 4th edition. Cambridge: Blackwell Science, 1996: 605-31.

4) Hansen ME, Valentine RJ, McIntire DD, et al. Age-related differences in the distribution of peripheral atherosclerosis: when is atherosclerosis truly premature? Surgery 1995; **118**: 834-9.

5) White JV. Lower extremity arterial disease: general considerations. In: Cronenwett JL, Johnston KW eds. Rutherford's Vascular Surgery, 7th edition. Philadelphia: Saunders, 2010: 1576-92.

6) Chen Q, Shi Y, Wang Y, et al. Patterns of disease distribution of lower extremity peripheral arterial disease. Angiology 2015; **66**: 211-8.

7) Fujihara M, Fukata M, Odashiro K, et al. Reduced plasma eicosapentaenoic acid-arachidonis acid ratio in peripheral artery disease. Angiology 2013; **64**: 112-8.

8) Fukuda S, Fujioka S, Hosaka S, et al. Relationship between arteriosclerosis obliterans and the ratio of serum eicosapentaenoic acid to arachidonic acid. Ann Thorac Cardiovasc Surg 2014; **20**: 44-7.

9) Sugiura T, Yoshikawa D, Ishii H, et al. Relation of omega-3 fatty acid and C-reactive protein to peripheral artery disease in patients with coronary artery disease. Heart Vessels 2014; **29**: 449-55.

10) Leng GC, Horrobin DF, Fowkes FG, et al. Plasma essential fatty acids, cigarette smoking, and dietary antioxidants in peripheral arterial disease. A population-based case-control study. Arterioscler Thromb Vasc Biol 1994; **14**: 471-8.

11) Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 2006; **83 Suppl**: 1505S-19S.

12) McGill HC Jr, McMahan CA, Herderick EE, et al. Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery. Arterioscler Thromb Vasc Biol 2000; **20**: 836-45.

13) Aboyans V, Lacroix P, Criqui MH. Large and small vessels atherosclerosis: similarities and differences. Prog Cardiovasc Dis 2007; **50**: 112-25.

14) Diehm N, Shang A, Silvestro A, et al. Association of cardiovascular risk factors with pattern of lower limb atherosclerosis in 2659 patients undergoing angioplasty. Eur J Vasc Endovasc Surg 2006; **31**: 59-63.

15) Kashiyama T, Ueda Y, Nemoto T, et al. Relationship between coronary plaque vulnerability and serum n-3/n-6 polyunsaturated fatty acid ratio. Circ J 2011; **75**: 2432-8.

16) Cawood AL, Ding R, Napper FL, et al. Eicosapentaenoic acid (EPA) from highly concentrated n-3 fatty acid ethyl esters is incorporated into advanced atherosclerotic plaques and higher plaque EPA is associated with decreased plaque inflammation and increased stability. Atherosclerosis 2010; **212**: 252-9.

17) Mori TA, Watts GF, Burke V, et al. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation 2000; **102**: 1264-9.

18) Itakura H, Yokoyama M, Matsuzaki M, et al. Relationships between plasma fatty acid composition and coronary artery disease. J Atheroscler Thromb 2011; **18**: 99-107.

19) Ueeda M, Doumei T, Takaya Y, et al. Association of serum levels of arachidonic acid and eicosapentaenoic acid with prevalence of major adverse cardiac events after acute myocardial infarction. Heart Vessels 2011; **26**: 145-52.

20) Domei T, Yokoi H, Kuramitsu S, et al. Ratio of serum n-3 to n-6 polyunsaturated fatty acids and the incidence of major adverse cardiac events in patients undergoing percutaneous coronary intervention. Circ J 2012; **76**: 423-9.

21) Erkkilä AT, Matthan NR, Herrington DM, et al. Higher plasma docosahexaenoic acid is associated with reduced progression of coronary atherosclerosis in women with CAD. J Lipid Res 2006; **47**: 2814-9.

22) Iso H, Kobayashi M, Ishihara J, et al. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-based (JPHC) study Cohort I. Circulation 2006; **113**: 195-202.

23) Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. Lancet 2007; **369**: 1090-8.

24) Ishikawa Y, Yokoyama M, Saito Y, et al. Preventive effects of eicosapentaenoic acid on coronary artery disease in patients with peripheral artery disease—subanalysis of the JELIS trial—. Circ J 2010; **74**: 1451-7.

25) Mori TA, Bao DQ, Burke V, et al. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. Hypertension 1999; **34**: 253-60.

26) Woodman RJ, Mori TA, Burke V, et al. Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. Atherosclerosis 2003; **166**: 85-93.

27) Smith FB, Lee AJ, Fowkes FG, et al. Variation in cardiovascular risk factors by angiographic site of lower limb atherosclerosis. Eur J Vasc Endovasc Surg 1996; **11**: 340-6.