Relationship between n-3 Polyunsaturated Fatty Acids and Extent of Vessel Disease in Patients with ST Elevation Myocardial Infarction

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Summary

A relationship between serum polyunsaturated fatty acids (PUFAs) and cardiovascular disease has been reported; however, the existence of a relationship between serum PUFAs and extent of vessel disease (VD) in patients with ST elevation myocardial infarction (STEMI) remains unclear.

Between July 2011 and June 2015, 866 consecutive STEMI patients underwent emergent percutaneous coronary intervention, 507 of whom were enrolled and classified into three groups according to the initial angiograms: 1VD, 294 patients; 2VD, 110 patients; and 3VD/left main trunk disease (LMTD), 103 patients. Serum levels of PUFAs, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid, and other laboratory data during hospitalization were evaluated.

The serum EPA level in the 3VD/LMTD group was significantly lower than that in the 1VD group (55.5 ± 22.1 versus 66.2 ± 28.7 , $P = 0.002$) and was slightly lower than that in the 2VD group (55.5 ± 22.1 versus 65.2 ± 28.9 , $P = 0.0167$). Multivariate adjustment analysis revealed that age ≥ 70 years (odds ratio, 1.72; 95% confidence interval, 1.03-2.89; $P = 0.038$) and a low serum EPA level (odds ratio, 0.98; 95% confidence interval, 0.99-1.00; $P = 0.023$) were independent risk factors for 3VD/LMTD, while a low serum DHA level was not.

A low serum EPA level may be more strongly related than a low serum DHA level to the extent of VD in STEMI patients. Age ≥ 70 years and a low serum EPA level may be independent risk factors for 3VD/LMTD.

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Key words: Eicosapentaenoic acid, Docosahexaenoic acid, Multivessel disease, Acute coronary syndrome, Cardiovascular disease

An association between fish intake and risk of cardiovascular disease has been demonstrated in several epidemiological studies and clinical trials.¹⁻⁴⁾ Fish oil includes n-3 polyunsaturated fatty acids (PUFAs), among which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to be inversely associated with the extent of atherosclerosis, cardiovascular events, and mortality.⁵⁻¹²⁾

using integrated backscatter intravascular ultrasound,¹³⁾ and Urabe, *et al* reported that a low serum EPA level is associated with the presence and extent of high-risk plaques detected by coronary computed tomography angiography in patients receiving statin therapy.¹⁴⁾ However, little is known about the relationship between n-3 PUFAs and coronary atherosclerosis in patients with acute coronary syndrome, especially in patients with ST elevation myocardial infarction (STEMI).

We investigated the relationship between serum PUFAs, namely EPA, DHA, and arachidonic acid (AA), and the extent of vessel disease (VD) as a measure of coronary atherosclerosis in STEMI patients.

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Recent studies have demonstrated a direct relationship between n-3 PUFAs and the extent of coronary atherosclerosis. Amano, *et al* showed that low levels of n-3 PUFAs are associated with lipid-rich plaques detected by

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Figure 1. Study flow chart. STEMI indicates ST elevation myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass grafting, PUFA, polyunsaturated fatty acid; VD, vessel disease; and LMTD, left main trunk disease.

Methods

Study sample and study protocol: This study was designed as a single center, cross-sectional study to assess the relationship between n-3 PUFAs and the extent of VD as a measure of atherosclerosis in STEMI patients. The diagnosis of STEMI was based on the Universal Definition of Myocardial Infarction,¹⁵⁾ including a rise of cardiac biomarker values with symptoms of ischemia and new or presumably new significant ST segment changes. The exclusion criteria were n-3 PUFA agent administration, no severe cardiac damage (creatinine phosphokinase level remaining not greater than 3 times the upper limit of the normal range), embolic myocardial infarction, history of percutaneous coronary intervention or coronary artery bypass graft surgery, and in-hospital death. A total of 866 consecutive STEMI patients underwent emergent percutaneous coronary intervention between July 2011 and June 2015. Of these 866 patients, 507 were enrolled in our study. The 507 patients were divided into 3 groups according to the initial angiograms: 1VD group, 294 patients; 2VD group, 110 patients; and 3VD/left main trunk disease (LMTD) group, 103 patients (Figure 1). The LMTD group included LMTD patients with or without any other VD. One culprit lesion was counted as one VD, and the non-culprit VD was defined as more than 75% stenosis in one angiographic view or more than 50% stenosis in two or more angiographic views.

To assess the inter-observer variability, the angiographic diagnosis of the extent of VD or LMTD required an independent view and the agreement of 2 independent cardiologists (T.S. and K.K.) who were blinded to the clinical and procedural data. Furthermore, 1 of the 2 observers (T.S.) evaluated all the angiograms again after the initial evaluation to assess the inter-observer variability for the diagnosis of the extent of VD or LMTD. In cases of

disagreement, the evaluation of a third observer (H.S.) was obtained and the final decision on the diagnosis of the extent of VD or LMTD was made by consensus.

This study was conducted with the approval of the institutional ethics committee (approval no. 2059), and we obtained informed consent from all enrolled study patients to participate in this clinical study.

Data collection and laboratory examinations: Laboratory data including data for serum PUFA levels, lipid profiles, hemoglobin A1c (HbA1c), and other biochemical markers were collected routinely within 8 days after admission. Data that were unavailable within 8 days after admission were collected before discharge. Serum levels of PUFAs, including those of EPA, DHA, and AA were assayed by gas chromatography at an external laboratory (SRL, Tokyo). Lipid profiles included low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. The estimation of HbA1c level was based on the method certified by the National Glycohemoglobin Standardization Program. The estimated glomerular filtration rate (eGFR) was calculated by the Japanese equation using serum creatinine level, age, and sex as follows: $eGFR \text{ (mL/minute/1.73 m}^2\text{)} = 194 \times Cr^{-1.094} \times \text{age}^{-0.287} \times 0.739 \text{ for women}$.¹⁶⁾

Other variables: We checked all medications before the onset of STEMI, including statins, antihypertensive agents, antiplatelet agents, and antidiabetic agents. Antihypertensive agents included angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, calcium channel blockers, and β blockers. Antiplatelet agents included aspirin, clopidogrel, ticlopidine, and cilostazol. Prasugrel and ticagrelor had not been approved in Japan during the study period.

Statistical analysis: Continuous, normally distributed data are expressed as the mean $\pm$ SD, and skewed data are expressed as the median and the first and third quartiles.

Table I. Baseline Patient Characteristics

	Overall (507 patients)	1VD (294 patients)	2VD (110 patients)	3VD+LMTD (103 patients)	<i>P</i>
General characteristics					
Age, years	68.1 ± 12.1	66.9 ± 12.6	69.1 ± 10.7	70.4 ± 12.0	0.02
Men	384 (75.7)	222 (75.5)	83 (75.5)	79 (76.7)	0.97
Body mass index, kg/m ²	23.9 ± 3.5	23.9 ± 3.6	24.1 ± 3.5	23.5 ± 3.0	0.46
Cardiogenic shock on arrival	48 (9.5)	18 (6.1)	7 (6.4)	23 (22.3)	< 0.0001
Culprit Vessels					
LAD	242 (47.7)	155 (52.7)	48 (43.6)	39 (37.9)	< 0.0001
LCX	49 (9.7)	24 (8.2)	15 (13.6)	10 (9.7)	
LMT	9 (1.8)	0 (0)	0 (0)	9 (8.7)	
RCA	207 (40.8)	115 (39.1)	47 (42.7)	45 (43.7)	
Risk Factors					
Diabetes mellitus	160 (31.6)	84 (28.6)	35 (31.8)	41 (39.8)	0.11
Hyperlipidemia	225 (44.4)	130 (44.2)	43 (39.1)	52 (50.5)	0.25
Hypertension	317 (62.5)	176 (59.9)	74 (67.3)	67 (65.1)	0.33
Current smoker	179 (35.3)	111 (37.8)	37 (33.6)	31 (30.1)	0.34
Family history	58 (11.4)	38 (12.9)	13 (11.8)	7 (6.8)	0.21
Hemodialysis	5 (1.0)	1 (0.3)	2 (1.8)	2 (1.9)	0.22
Medications before admission					
Statins	104 (20.5)	58 (19.7)	25 (22.7)	21 (20.4)	0.80
Antihypertensive agents	218 (43.0)	118 (40.1)	51 (46.4)	49 (47.6)	0.31
Antiplatelet agents	51 (10.1)	29 (9.9)	8 (7.3)	14 (13.6)	0.31
Antidiabetic agents	97 (19.1)	50 (17.0)	20 (18.2)	27 (26.2)	0.13
No medication	204 (40.2)	131 (44.6)	40 (36.4)	33 (32.0)	0.052
Laboratory data					
LDL-cholesterol, mg/dL	100.7 ± 31.4	103.3 ± 31.7	97.5 ± 29.9	96.6 ± 31.6	0.08
HDL-cholesterol, mg/dL	38.8 ± 9.5	39.3 ± 10.1	37.3 ± 8.7	37.0 ± 8.2	0.005
Triglycerides, mg/dL	110.1 ± 46.2	110.1 ± 46.2	109.6 ± 46.7	110.7 ± 45.9	0.98
Hemoglobin A1c, %	5.8 (5.5-6.5)	5.8 (5.5-6.3)	5.8 (5.5-6.5)	6.0 (5.6-6.8)	0.049
Creatinine, mg/dL	0.81 (0.69-1.0)	0.80 (0.68-0.96)	0.82 (0.69-1.01)	0.87 (0.72-1.1)	0.03
eGFR	69.2 ± 23.0	71.4 ± 22.5	68.0 ± 20.5	64.4 ± 26.1	0.02
BNP, pg/mL	116.5 (45.4-266.4)	97.5 (39.5-201.7)	145.1 (56.1-302.9)	188.2 (54-463.3)	0.0002
Hemoglobin, g/dL	12.7 ± 1.9	12.9 ± 1.9	12.6 ± 2.0	12.3 ± 1.9	0.03
max CPK, IU/L	2747 (1638-4416)	2839 (1578.3-4411.5)	2824 (1843.3-4323.8)	2464 (1605-4974)	0.80

Values are means (standard deviations) or medians (interquartile ranges) and are expressed as numbers (%) unless otherwise specified. Differences between the three groups were calculated by the one-way ANOVA test. LAD indicates left anterior descending coronary artery; LCX, left circumflex coronary artery; LMT, left main trunk; RCA, right coronary artery; LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; BNP, brain natriuretic peptide; and CPK, creatine phosphokinase.

Continuous variables were compared using the one-way ANOVA test or the Kruskal-Wallis test based on the distribution. Categorical variables were compared using the chi-square test or Fisher's exact test, as appropriate. Serum levels of PUFAs in the 3 groups were compared using the Bonferroni correction for multiple comparisons. Basically, *P*-values of < 0.05 were considered to be statistically significant. By using the Bonferroni correction, *P*-values of < 0.016 were considered to be statistically significant. Multivariate logistic regression analysis was performed to identify independent risk factors for 3VD/LMTD. Variables for multivariable analyses were selected if they were empirically known to have an influence on atherosclerosis. The cut-off value of age was set to 70 years in accordance with daily practice, and that of eGFR was set to 60 mL/minute/1.73 m² to select patients having moderate to severe chronic kidney disease. Independent variables are expressed as the odds ratio with the 95% confidence interval. JMP 9 (SAS Institute Inc., Cary, NC, USA) was used for all statistical calculations.

Results

Study sample: The baseline patient characteristics are shown in Table I. There were no significant differences between the 3 groups in risk factors and in medications before admission. The extent of VD showed correlations with HDL-C, HbA1c, eGFR, brain natriuretic peptide, hemoglobin, and serum EPA level. Correlations of the extent of VD with age and cardiogenic shock were also found.

A total of 480 (94.7%) of the 507 patients underwent in-hospital statin therapy.

Serum PUFA ratios by sex and age groups: The ratios of serum EPA/AA and DHA/AA varied significantly in the male patients according to the age groups and were lower in younger men but did not vary significantly in the female patients according to the age groups (Figure 2).

Serum levels of PUFAs and extent of VD: Serum EPA level in the 3VD/LMTD group was significantly lower than that in the 1VD group (55.5 ± 22.1 versus 66.2 ± 28.7, *P* = 0.002) and tended to be lower than that in the 2VD group (55.5 ± 22.1 versus 65.2 ± 28.9, *P* = 0.0167).

Figure 2. Ratios of serum polyunsaturated fatty acids by sex and age groups. EPA indicates eicosapentaenoic acid; DHA, docosahexaenoic acid; and AA, arachidonic acid.

Figure 3. Relationship between serum polyunsaturated fatty acids and extent of vessel disease in patients with ST elevation myocardial infarction. **A:** EPA, **B:** DHA, **C:** AA, **D:** EPA/AA ratio, **E:** DHA/AA ratio. Differences between the 3 groups were calculated by the one-way ANOVA test, and Bonferroni's correction was used as a post-hoc analysis ($P < 0.016$). See the legend of Figure 2 for the definitions of abbreviations.

(Figure 3A). Serum DHA level tended to be lower in the 3VD/LMTD group than in the 1VD group (133.7 ± 38.7 versus 146.2 ± 43.8 , $P = 0.02$) (Figure 3B). Serum AA level was not significantly different between the 3 groups (Figure 3C). The ratios of serum EPA/AA and DHA/AA were not significantly different between the 3 groups (Figure 3D, E).

In addition, between patients with 3VD and those

with LMTD, the serum EPA (55.9 versus 54.3 , $P = 0.75$), serum DHA (136.6 versus 126.2 , $P = 0.27$), and serum AA levels (171.1 versus 173.3 , $P = 0.82$) were similar.

Independent risk factors for 3VD/LMTD by multivariable analysis: Multivariable analysis showed that age ≥ 70 years (odds ratio, 1.70; 95% confidential interval, 1.02-2.86; $P = 0.041$) and a low serum EPA level (odds ratio, 0.98; 95% confidential interval, 0.97-1.00; $P = 0.023$)

Table II. Independent Determinants of 3VD/LMTD by Multivariable Analysis

Variable	Odds ratio	95% confidence interval	P
Age $\geq$ 70	1.76	1.05-2.97	0.03
Diabetes mellitus	1.36	0.82-2.26	0.23
HDL-C	0.98	0.96-1.01	0.24
Statin before admission	1.08	0.59-1.92	0.79
eGFR $\leq$ 60	1.31	0.77-2.19	0.32
EPA	0.98	0.97-1.00	0.024
DHA	1.00	0.99-1.01	0.94

3VD indicates 3-vessel disease; LMTD, left main trunk disease; HDL-C, high-density lipoprotein cholesterol; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; and DHA, docosahexaenoic acid.

were independent risk factors for 3VD/LMTD, while a low serum DHA level was not (Table II).

Discussion

Our data demonstrated that the serum EPA level was inversely associated with the extent of VD in STEMI patients. Whether the serum DHA level is inversely associated with atherosclerosis is still controversial,^{8,9,17,18)} but multivariable analysis of the data in our study revealed that it was not associated with the extent of VD.

Because the effects of serum EPA are opposite to those of serum AA, the ratios of serum EPA/AA and DHA/AA have been reported to be correlated with inflammation in the human body and to be associated with cardiovascular events.^{5,9,19-21)} In our study, however, they were not significantly different between the 3 groups, which may be attributed to the distribution of the serum AA level as shown in Figure 3C. We speculate that the dietary habits of the patients affect the distribution of the serum AA level. Because the average age of patients with 1VD was significantly younger, they are likely to have more Western-style meals which often include more AA. However, it remains unclear due to the lack of data on dietary habits of the study sample before the onset of STEMI.

Serum levels of PUFAs are known to be differently distributed according to sex and age, reflecting dietary habits and age-related changes, and residential areas with different levels of fish consumption,²²⁻²⁶⁾ but their tendencies in STEMI patients remain unclear. In our study, the ratios of serum EPA/AA and DHA/AA in STEMI patients significantly varied in the male patients according to the age groups and were lower in younger men, as is the case for healthy people.²²⁾ The distribution of the ratios of serum PUFAs was not affected by the occurrence of STEMI. The fact that the ratios of serum EPA/AA and DHA/AA did not significantly vary in the female patients according to the age groups can be attributed to the small number of women. Because serum levels of PUFAs may be affected by regional characteristics, this tendency may be peculiar to the area around our hospital.

Some randomized controlled trials have shown that supplementation of serum PUFAs may reduce cardiovascular events even during statin therapy,^{27,28)} but other trials

have shown that such supplementation may not reduce cardiovascular events.^{29,30)} The discrepancy in the results of those trials may be due to differences in the amount of EPA contained in supplementation and the study patients. Administration of sufficient amounts of highly purified EPA may be beneficial for patients with severe coronary lesions.

Limitations: Our study has the following major limitations: 1) It was a single-center, cross-sectional study, 2) some patients with 3VD/LMTD might have been excluded from the study sample due to the exclusion of in-hospital deaths, 3) lipid profiles might have been influenced by in-hospital statin therapy because the timing of laboratory data collection was not on admission, 4) 3VD patients were combined with LMTD patients, 5) dietary habits before admission were not taken into consideration, and 6) we have evaluated vessel disease based on the severity of coronary artery stenosis, including both culprit and non-culprit lesions.

It remains to be studied whether administration of sufficient amounts of highly purified EPA is beneficial to patients with severe coronary lesions.

Conclusion

Low serum EPA levels may be more strongly related than low serum DHA levels to the extent of VD in STEMI patients. Age $\geq$ 70 years and a low serum EPA level may be independent risk factors for 3VD/LMTD.

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Disclosures

Conflicts of interest: Takenobu Shimada received speaking fees from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan. Other authors declare that they have no competing interests.

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