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Erythrocyte Membrane and Plasma Fatty Acids Composition in Patients with Coronary Heart Disease Requiring Percutaneous Angioplasty

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Authors' contributions

This work was carried out in collaboration among all authors. Author TA had an initial research concept and performed data acquisition. Authors TY and MF performed patients management and enrollment. Author TM contributed to the manuscript writing and was a corresponding author. Authors SH and SM contributed to the biochemical assay. Author TF made advice on study protocol. Author KA supervised team collaboration as a team leader. All authors read and approve the final manuscript.

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ABSTRACT

Aims: Atherosclerosis is associated with oxidative stress and fatty acid composition plays a critical role in vascular endothelial dysfunction and injury leading to the coronary atherosclerotic progression. However, the correlation between the fatty acid profile and coronary atherosclerosis is debatable. The goal of this study is to assess the erythrocyte membrane and plasma fatty acid composition in patients with coronary heart disease.
Methods: The erythrocyte membrane and plasma distributions of fatty acids were quantified in patients with coronary heart disease (n = 30, group A) which needs both intensive medication and

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elective percutaneous coronary intervention and age-matched controls (n = 38, group B) using high-performance liquid chromatography combined with evaporative light scattering detection method. Baseline data were extracted from medical records.
Results: Logistic regression analysis demonstrated that hypoalbuminemia (p = 0.010) and HbA1c (p = 0.005) are associated with required percutaneous coronary intervention. Although appropriate logistic regression model for percutaneous coronary intervention could not be obtained by incorporating fatty acid components, percutaneous coronary intervention was correlated mostly to the increased oleic acid and decreased stearic acid in both erythrocyte membrane and plasma in receiver-operating characteristic analysis.
Conclusion: This single-center, cross-sectional study indicated that erythrocyte membrane and plasma fatty acids have a potential impact on the coronary atherosclerotic progression which requires coronary intervention. Longitudinal studies are necessary to clarify the clinical role of fatty acids distribution as a novel atherogenic marker.

Keywords: Coronary heart disease; percutaneous angioplasty; erythrocyte membrane; fatty acids.

1. INTRODUCTION

Coronary heart disease (CHD) remains a leading cause of mortality and morbidity in many countries. CHD includes angina pectoris, acute coronary syndrome and sudden cardiac death. The pathogenic role of oxidative stress on CHD includes the progression of inflammatory process in endothelial layer, coronary plaque formation, and plaque rupture [1,2]. Oxidative stress alters the erythrocyte membrane and plasma phospholipids and fatty acids distributions [3]. Oxidative damage impairs the erythrocytes deformability leading to the disturbed coronary microcirculation [4,5]. Erythrocyte membrane is prone to be damaged by oxidative stress. Irrespective of saturated or unsaturated, many kinds of fatty acids are incorporated to erythrocyte membrane phospholipids. Therefore, erythrocyte membrane fatty acid profile is influenced by redox conditions [6].

In patients with CHD, percutaneous coronary intervention (PCI) is a widely acceptable technique to keep the culprit lesion patent, and to prevent future cardiac events and to improve the long-term outcome. Recently, we found that erythrocyte membrane and plasma plasmalogens were reduced in patients with CHD requiring elective PCI [7]. This unique phospholipid contains many amounts and kinds of unsaturated fatty acids [8]. Therefore, this finding is compatible with that plasmalogens are unique antioxidative substance [9]. The present study investigated the fatty acid profiles in erythrocyte membrane and plasma which were obtained from CHD patients. These patients underwent elective PCI and the same subjects as those in our recent study [7].

2. METHODOLOGY

2.1 Selection of Subjects

This study was a case-control study composed of two subjects' groups and performed from February to August 2016. This study was conducted according to the Declaration of Helsinki (2008). This study enrolled 30 patients with CHD (72.3 ± 7.9 years, 6 women and 24 men; group A) and 38 age-matched controls (71.6 ± 5.1 years, 25 women and 13 men; group B). We excluded patients with hemodialysis, dementia and cancer. Blood was sampled in the morning after the overnight fasting. We measured blood pressure (BP) of patients at sitting position by sphygmomanometer after taking a few minutes' rest. Body weight (kg) divided by square of height (m^2) induces body mass index (BMI). Hypertension was defined as systolic BP ≥ 130 mmHg, diastolic BP ≥ 85 mmHg and/or the prescription of antihypertensive agents. Diabetes was defined as a fasting blood glucose concentration ≥ 126 mg/dl, a casual blood glucose concentration ≥ 200 mg/dl, hemoglobin A1c (HbA1c) ≥ 6.5% and/or the prescription of antidiabetic agents. HbA1c was based on the National Glycohemoglobin Standardization Program (NGSP). Dyslipidemia was defined as low-density lipoprotein (LDL) cholesterol ≥ 140 mg/dl, high-density lipoprotein (HDL) cholesterol < 40 mg/dl and/or the prescription of lipid-lowering agents [10,11].

Patients with CHD in the Heart Center of the Kyushu University Hospital (Fukuoka, Japan) undergoing PCI were reviewed (group A). Patients with CHD were those with stable effort angina pectoris (n = 18) and those with old myocardial infarction (n = 12). There were no

smokers, i.e., smoking had been prohibited before the enrollment into this study. Coronary risk factors were prevalent in patients of group A. Hypertension (n = 22, 73.3%), diabetes (n = 13, 43.3%) and dyslipidemia (n = 16, 53.3%) were found in these patients with CHD. The control of risk factors was under the discretion of the treating physicians [7]. Hypertension was treated by antihypertensive drugs such as Ca blockers (n = 11, 36.7%), angiotensin receptor blockers (n = 12, 40%) and β-blockers (n = 15, 50%), although combined antihypertensive medication was frequent (n = 12, 40%). Statin was prescribed (n = 17, 56.7%), and highly purified agents of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) were prescribed (n = 9,30%). Many of them were prescribed with antiplatelet agents such as aspirin, clopidogrel, prasugrel, and the combination of these (n = 23, 76.7%).

Group B included the outpatient of BOOCS Clinic (Fukuoka, Japan) visiting monthly for health screening or controlling of mild hypertension or dyslipidemia. Subjects in group B were prescribed with less medication than those in group A, i.e., Ca blockers (13.2%), angiotensin receptor blockers (5.3%) and statin (7.9%) were prescribed. EPA/DHA agents were not prescribed. There were no smokers in this group. This study was conducted according to the current ethical standards of each institutional and/or national research committee, i.e., we obtained signed informed consent from each subject after the admission and before the PCI in group A or at the enrollment into the study in group B.

2.2 Measurements of Plasma Fatty Acids

Venous blood was sampled by disposable syringe which contained heparin. Sample was packed and cooled immediately in ice bath. Sample was kept in refrigerator and processed within 48 hours. Plasma was separated by centrifugation at 1,000 x g for 5 minutes at 4ºC and kept at -80ºC until use. Phospholipids in plasma were quantified after the treatment with phospholipase A_1 (PLA_1) [12]. PLA_1 was purchased from Sigma-Aldrich Co. (Tokyo, Japan) and Mitsubishi Kagaku Foods Co. (Tokyo, Japan). PLA_1 was diluted with an equal volume of 0.1 M citrate buffer (pH 4.5). Twenty µl of the diluted PLA_1 was added to 80 µl of plasma and incubated at 45ºC for 60 minutes.

After the treatment with PLA_1, lipid extraction was conducted by adding 800 µl of *n*-hexane/isopropanol (3:2, v/v) to the PLA_1-treated plasma. After mixing vigorously, it was sonicated for 5 minutes. Thereafter, 400 µl of Na_2SO_4 solution was added and left for 5 minutes, and 400 µl of hexane layer was transferred to a new conical Eppendorf tube. Then, 400 µl of hexane/isopropanol (7:2, v/v) was added to the lower phase and mixed vigorously, and the hexane layer (300 µl) was recovered. The combined hexane layer was dried by means of N_2 gas and stored at -30ºC until analysis. Phospholipid including ether phospholipids was classified by high performance liquid chromatography (HPLC). HPLC was composed of an Agilent 1100 equipped with a four-solvent delivery system, a degasser and automatic injector. The column used in HPLC was a LiChrosphere 100 Diol (250 x 2 mm), 5 µm (Merck, Germany). The column temperature was 50ºC and flow rate was 0.4 ml/min. Mobile phase A was *n*-hexane/2-propanol/acetic acid (82:17:1, v/v/v) with 0.08% triethylamine (TEA), and mobile phase B was 2-propanol/water/acetic acid (85:14:1, v/v/v) with 0.08% TEA. The lipid extract after PLA_1 treatment of plasma was reconstituted with 200 µl hexane/isopropanol (3:2, v/v), and 20 µl was applied to the HPLC. This HPLC was combined with evaporative light scattering detector (ELSD). The phospholipid classes were detected by ELSD (Agilent 1900 Infinity) with the following settings: evaporation temperature, 60ºC; sensitivity gain, 6; flow rate of N_2 gas, 1 l/min. Nebulizer temperature was 30ºC. HPLC-ELSD system was validated and linearity for calibration was updated periodically. To date, linear regression curves for calculation of phospholipid concentration from chromatographic peak area showed $R^2 > 0.97$ [12].

Phospholipids were collected by a single run of HPLC and used for the analysis of fatty acids composition [3]. Each phospholipid fractions derived from plasma was dried using a stream of N_2 gas and was hydrolyzed with 1 ml of 0.5 M KOH in 70% methanol at 70ºC for 60 min. The fatty acids were extracted with 2 ml hexane after acidification with 0.3 ml of 2N HCl. The hexane solution was washed with 1 ml of water. The hexane was evaporated by means of N_2 gas stream. SM was initially hydrolyzed with 2N HCl in methanol at 100ºC for 5 hours, and fatty acids were extracted with chloroform. Then, dried fatty acids were processed as other phospholipid classes. The fatty acids were labeled with 9-

anthryldiazomethane (ADAM) by mixing with 200 µl of 0.05% ADAM in methanol and the mixture was left in dark at room temperature (22 ± 3ºC) for more than 2 hours. An aliquot (10-20 µl) was injected to HPLC system after the filtration through a filter with 0.2 µm pore size. A TSK-GEL ODS-80Ts column (250 x 4.6 mm, 5 µm; Tosoh, Tokyo, Japan) was used for separation of fatty acids. Each fatty acid labeled with ADAM was detected with a FS-8020 fluorescence detector (Tosoh). Wavelength setting was 418 nm for emission and 365 nm for extraction. The detector was connected to the HP 35900 interface and the computer with HPLC ChemStation (Hewlett-Packard, Tokyo, Japan). Ternary gradient elution was used, and the mobile phase contained acetonitrile, methanol and water, although acetonitrile was maintained at 10% throughout the elution. Methanol concentration was 82% at 0 min, 83% at 18 min, 84% at 36 min, 86% at 36.1 min, 90% at 45 min and 90% at 60 min, respectively. The turnaround time was 70 min, the flow rate was 1 ml/min, and the column temperature was 50ºC. Percentage of individual fatty acid depends on the peak area of chromatographic elution.

2.3 Measurements of Erythrocyte Membrane Fatty Acids

Extraction of erythrocyte membrane phospholipids was carried out based on the previously reported method [13]. Briefly, after centrifugation at 1,000 x *g* for 5 minutes at 4ºC, plasma and buffy coat were aspirated carefully from venous blood. Packed erythrocytes were washed three times in cold isotonic saline at 1,000 x *g* for 5 minutes at 4ºC. A small portion of the supernatant was removed carefully at each washing. Then, erythrocytes were hemolyzed with hypotonic buffer (10 mM Tris-HCl, pH 7.4) and were centrifuged at 25,000 x *g* for 20 min at 4ºC. This procedure was repeated four times to remove hemoglobin completely. The isolated erythrocyte membrane was kept at -80ºC. Lipids from erythrocyte membrane were extracted with chloroform/methanol (1:2, v/v) method and total lipids were dried using the N_2 gas stream. After the lipids were re-suspended in hexane/isopropanol (3:2, v/v), erythrocyte membrane phospholipid classes were separated by means of the HPLC-ELSD system. This system collects all the classes of phospholipid in erythrocyte membrane by a single run of HPLC. The measurement of erythrocyte membrane fatty acid composition was the same as that of plasma fatty acid composition as aforementioned [3]. All experiments were performed at room temperature (22 ± 3ºC).

2.4 Data Analyses

Sample size was calculated to satisfy the 90% power and an α error of 0.05. Calculated sample size was ≥ 68 cases, which was based on the preliminary study concerning erythrocyte membrane phospholipids distribution [14]. Discrete data between the two groups were compared by the chi-square (χ^2) test or the Fisher's exact test. Yates' correction was used, if necessary. Continuous data were expressed as means ±SD. Kolmogorov-Smirnov test was used for data distribution showing normality or not. Normally distributed data were compared by unpaired Student's *t* test. Data without normality were compared by Mann-Whitney *U* test. Logistic regression analysis was used to seek the contributory factors to PCI. Criteria for incorporating into the regression model were statistical significance or otherwise meaningful variables. The logistic models showing adjusted coefficients of determination (R^2) ≥ 0.550 were accepted. Finally, receiver-operating characteristic (ROC) analyses were performed to seek the factors contributing to PCI. ROC curves showing area under the curves (AUC) ≥ 0.650 were acceptable. These analyses were conducted using Bell Curve for Excels version 2.12 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Ninety-five% confidence of interval (CI) was expressed. Differences with two-sided $p < 0.05$ were considered to be significant.

3. RESULTS

3.1 Baseline Characteristics of Subjects

The baseline characteristics of the enrolled subjects are detailed in our current study [7]. PCI in all the patients of group A was successful. Although gender ratio in group A (6 females and 24 males) differed significantly ($p < 0.001$) from the ratio in group B (25 females and 13 males), there were no significant differences in age and BMI distributions between the two groups. Although hypertension, dyslipidemia and diabetes in group A were prevalent relative to those in group B ($p < 0.001$), systolic BP in group A did not differ from that in group B, and diastolic BP in group A was lower than that in group B ($p = 0.003$). HbA1c in group A was significantly greater than that in group B ($p < 0.001$), but average HbA1c in group A (6.2 ± 1.6%) was

Table 1. Relative plasma fatty acid components in the two groups

	Group A (*n* = 30)	Group B (*n* = 38)	*p* value
EPA (%)	2.02 ± 1.20	1.66 ± 0.96	0.292
DHA (%)	3.43 ± 1.18	2.11 ± 1.64	< 0.001
AA (%)	6.12 ± 1.07	3.86 ± 1.81	< 0.001*
EPA/AA	0.33 ± 0.19	0.56 ± 0.61	0.007
Linolenic acid (%)	1.35 ± 0.45	6.25 ± 1.84	< 0.001
Linoleic acid + Palmitoleic acid (%)	23.01 ± 1.98	23.48 ± 1.92	0.292
Oleic acid (%)	23.99 ± 2.74	21.85 ± 2.70	0.002*
Palmitic acid (%)	29.85 ± 2.31	28.98 ± 2.12	0.057
Stearic acid (%)	10.22 ± 1.46	11.82 ± 1.37	< 0.001*

**Data showing normality. Percentages indicate relative fatty acid components based on the chromatographic peak area (%). AA, arachidonic acid; DHA, dochosahexaenoic acid; EPA, eicosapentaenoic acid*

Table 2. Relative erythrocyte membrane fatty acid components in the two groups

	Group A (*n* = 30)	Group B (*n* = 38)	*p* value
EPA (%)	2.20 ± 1.32	1.42 ± 0.48	0.007
DHA (%)	9.70 ± 1.76	10.12 ± 1.09	0.237*
AA (%)	17.31 ± 1.37	17.18 ± 1.11	0.675*
EPA/AA	0.13 ± 0.08	0.08 ± 0.03	0.014
Linoleic acid + Palmitoleic acid (%)	13.07 ± 1.62	14.24 ± 1.26	0.002*
Oleic acid (%)	14.27 ±0.93	13.46 ± 0.72	< 0.001*
Palmitic acid (%)	26.45 ±1.23	26.09 ±0.90	0.172*
Stearic acid (%)	17.01 ± 0.98	17.49 ± 0.62	0.017*

**Data showing normality. Percentages indicate relative fatty acid components based on the chromatographic peak area (%). Abbreviations are as in Table 1*

within the normal range in the criteria of NGSP. With respect to cholesterol profile, LDL and HDL cholesterol concentrations in group A were lower than the corresponding concentrations in group B ($p < 0.001$). Consequently, total cholesterol level in group A was lower than that level in group B ($p < 0.001$). The patients in group A tended to be mildly anemic ($p = 0.013$) and hypoalbuminemic ($p < 0.001$) than the subjects in group B. Moreover, the patients in group A showed renal impairment ($p = 0.020$) as compared with subjects in group B.

3.2 Plasma Contents of Fatty Acids

Plasma fatty acid distribution was analyzed in the two groups of patients by HPLC-ELSD system, and relative compositions of fatty acids were calculated according to each chromatographic peak area [12]. These outcomes were demonstrated in Table 1. Nine patients in group A (30%) but not in group B were prescribed with highly purified EPA/DHA agents. Relative content of EPA (C20:5n-3) in group A did not differ from that in group B, whereas relative plasma content of DHA (C22:6n-3) in group A was higher than that in group B ($p < 0.001$). Relative plasma content of arachidonic acid (AA, C20:4) in group A was higher than that in group B ($p < 0.001$). Consequently, the plasma ratio of EPA/AA in group A was less than that ratio in group B (p = 0.007). Plasma content of linolenic acid (C18:3) in group A was less than in group B ($p < 0.001$). Plasma content of stearic acid (C18:0) showed the same tendency ($p < 0.001$), whereas that of oleic acid (C18:1) showed the opposite tendency ($p = 0.002$).

3.3 Erythrocyte Membrane Contents of Fatty Acids

Erythrocyte membrane fatty acid distributions in group A was compared with those in group B by HPLC-ELSD system on the basis that relative content of the individual fatty acid corresponds to the peak chromatographic area [13]. Relative contents of erythrocyte membrane fatty acid components were detailed in Table 2. Relative EPA content in group A was greater than that in group B ($p = 0.007$), and hence the EPA/AA ratio showed the same tendency ($p = 0.014$) under the administration of the highly purified EPA/DHA agents to the limited patients in group A. There were significant intergroup differences in relative contents of other fatty acids, i.e., combined linoleic acid (C18:2) and palmytoleic acid (C16:1)

were greater in group B (p = 0.002), oleic acid (C18:1) was greater in group A (p < 0.001) and stearic acid (C18:0) was greater in group B (p = 0.017) than in the counterpart group.

3.4 Association of PCI with Medical Data

Association of PCI with medical data was investigated by logistic regression analyses, where medical data indicate background characteristics and laboratory data including fatty acid components. Logistic regression model was constructed as a clinical model, where background characteristics and standard clinical data were incorporated. On the other hand, regression model was constructed as a membrane model, where only erythrocyte membrane fatty acid profile was incorporated. Acceptable R^2 > 0.550 was obtained by clinical model alone as demonstrated in Table 3. PCI was associated significantly with HbA1c (p = 0.005) and inversely with serum albumin (p = 0.010) under R^2 = 0.695 (p < 0.001). Although gender ratio in group A (M:F = 24:6) was significantly (p < 0.001) different from that in group B (M:F = 13:25), gender was not a contributor to the required PCI at all.

3.5 Correlation between PCI and Fatty Acids

Among the fatty acid species, those influenced by medication (highly purified EPA/DHA agents) or showing discrepant results among erythrocyte membrane and plasma analysis were excluded, and the other fatty acids were analyzed by ROC analyses which was applied to all the enrolled subjects, and ROC curves showing AUCs ≥ 0.650 were accepted. Consequently, ROC curves concerning oleic acid and stearic acid remained. ROC curves for PCI by erythrocyte membrane and plasma oleic acid (C18:1) presented AUCs of 0.750 (95%CI = 0.633 – 0.867, p < 0.001) and 0.725 (95%CI = 0.602 – 0.848, p < 0.001), respectively (Fig. 1). Those curves for PCI by erythrocyte membrane and plasma stearic acid (C18:0) presented AUCs of 0.695 (95%CI = 0.563 – 0.826, p = 0.004) and 0.804 (95%CI = 0.694 – 0.913, p < 0.001), respectively (Fig. 2). In both Figures, AUC for plasma fatty acid content did not differ significantly from AUC for corresponding erythrocyte membrane fatty acid content (p = 0.722 for oleic acid and p = 0.117 for stearic acid).

4. DISCUSSION

The basic findings of this study are that erythrocyte membrane and plasma distributions of oleic acid in patients with CHD were greater than those distributions in controls, and that the opposite was found in stearic acid distributions. The clinical finding is that required PCI for such patients was associated with hypoalbuminemia and diabetic progression which reflects inflammatory and oxidative vascular damage even under intensive medication for coronary risk factors.

Nowadays, PCI is a standard technique to rescue the culprit lesion of the stenotic coronary artery, and medical management of coronary risk factors is a prerequisite for elective PCI. The CHD patients in this study are under the intensive control of coronary risk factors. This was evident in that lipid profile and BP in group A were favorable relative to those in group B. Intrinsic PUFAs are primary target of oxidative stress, and low EPA/AA ratio is associated with peripheral artery disease progression in our study [15], which was confirmed in this study even under the partial supplementation of EPA/DHA agents (Table 1). Relatively to PUFAs, general fatty acid profile as a coronary risk factor remains debatable. Conflicting results have been

Table 3. Logistic regression analysis for association of percutaneous intervention with baseline characteristics and clinical data

Covariates	β	Standardized β	95%CI	*p* value
Age (year)	0.063	0.396	-0.139 — 0.264	0.542
Gender	-1.003	-0.500	-4.030 — 2.024	0.516
BMI (kg/m^2)	0.226	0.624	-0.227 — 0.680	0.328
Albumin (g/dl)	-6.373	-2.658	-11.242 — -1.503	0.010
HDL cholesterol (mg/dL)	-0.035	-0.571	-0.113 — 0.044	0.390
Creatinine (mg/dl)	3.245	2.031	-3.732 — 10.223	0.362
HbA1c	3.416	2.936	1.037 — 5.795	0.005

Adjusted coefficient of determination (R^2) = 0.695 (p < 0.001). BMI, body mass index

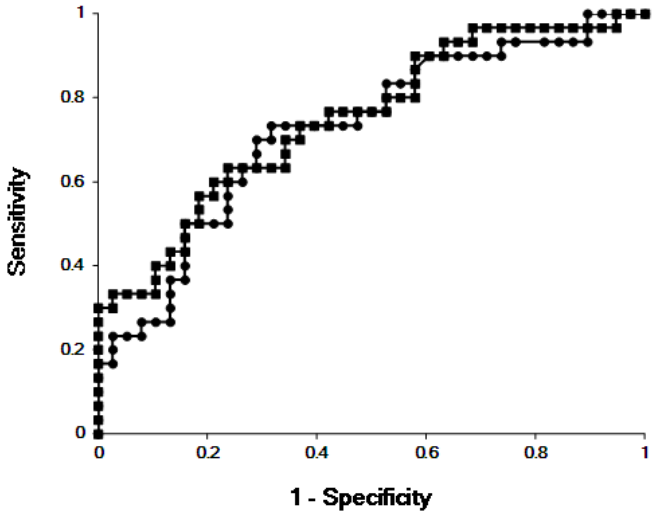

Fig. 1. ROC curves for the association of EVT with oleic acid

ROC curves for the association of EVT with plasma (solid circles) and erythrocyte (solid squares) oleic acid. AUC for the former is 0.725 (95%CI = 0.602 – 0.848, p < 0.001) and AUC for the latter is 0.750 (95%CI = 0.633 – 0.867, p < 0.001), showing no difference between the two AUCs (p = 0.722)

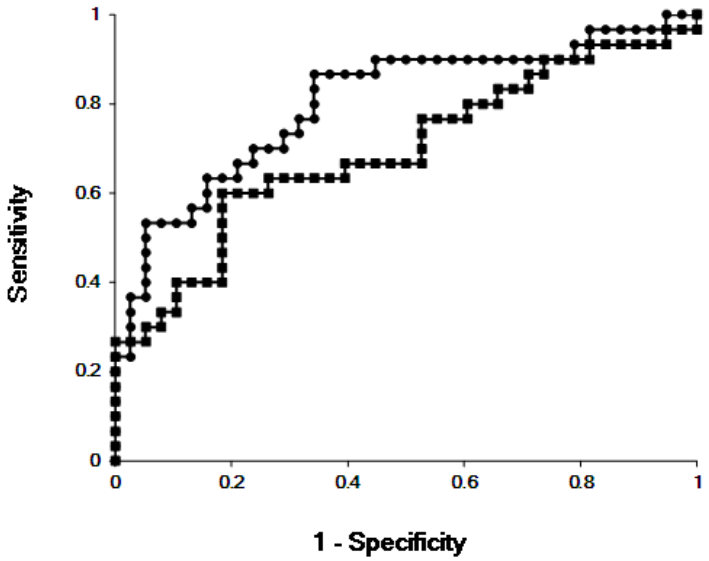

Fig. 2. ROC curves for the association of EVT with stearic acid

ROC curves for the association of EVT with plasma (solid circles) and erythrocyte (solid squares) stearic acid. AUC for the former is 0.804 (95%CI = 0.694 – 0.913, p < 0.001) and AUC for the latter is 0.695 (95%CI = 0.563 – 0.826, p = 0.004), showing no difference between the two AUCs (p = 0.117)

reported with respect to oleic acid (C18:1), i.e., this fatty acid is reported to play both promoting and preventive roles in future cardiac events [16,17]. We have currently reported that plasmalogen, a novel glycerophospholipid, is reduced in erythrocyte membrane and plasma in CHD patients enrolled in the present study [7]. Among choline plasmalogens, those containing oleic acid are inversely related to the severity of CHD [18], which is compatible with the present findings (Table 1).

The same is true with respect to stearic acid (C18:0), i.e., this saturated fatty acid is reported clinically to be negatively associated with proinflammatory cytokine of IL-6 and IL-8 [19]. In

contrast, this fatty acid is demonstrated to promote the differentiation of proinflammatory macrophages in obese mice [20]. The effects of individual fatty acid in plasma may differ from those of the same fatty acid supplemented orally. Discrepant findings in certain fatty acid are observed among the clinical and the basic investigations. Moreover, the competition among oleic acid and stearic acid is reported [21]. In this sense, the effects of fatty acids interacting mutually on the atherosclerotic progression seem complicated. It is unclear that the results of this study demonstrating greater distribution of oleic acid and lesser distribution of stearic acid in erythrocyte membrane and plasma of CHD patients undergoing PCI are the cause or the consequence of advanced coronary atherosclerosis.

This study acknowledges a few inherent limitations. First one is a study design. This is a cross-sectional, case-control study. There may be a potential bias to enroll and treat the patients. Because these were under the discretion of treating physicians. Consequently, treatment was optimized according to the guidelines leading to the intensive control of risk factors. Second one is a technical problem. Nowadays, the combination of liquid chromatography-tandem mass spectrometry (LC/MS/MS) is a standard technique to quantify the plasma ether phospholipids [12]. However, LC/MS/MS is time-consuming and not practical in our laboratory. Fatty acid composition was quantified in this study by HPLC-ELSD technique, which assays phospholipids and fatty acids separately, but authentic outcomes were obtained by this practical technique in our recent studies [22-25], Third limitation is lack of longitudinal study. Successful PCI is reported to attenuate the ongoing oxidative stress [26], and hence comparison of fatty acid compositions before and after the successful PCI is intriguing.

5. CONCLUSION

The present study demonstrated that erythrocyte membrane and plasma distributions of oleic acid and stearic acid in CHD patients are altered relative to those in controls. The required PCI for such patients was associated most with hypoalbuminemia and diabetic progression reflecting inflammatory and oxidative damages even under the intensive medication to control the coronary risk factors. Further longitudinal studies are required to focus on the ideal fatty acid balance to avoid the vascular damage and coronary atherosclerotic progression.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

This study was conducted according to the current ethical standards of each institutional and/or national research committee, i.e., we obtained signed informed consent from each subject after the admission and before the PCI in group A or at the enrollment into the study in group B.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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