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Reverse association of omega-3/omega-6 polyunsaturated fatty acids ratios with carotid atherosclerosis in patients on hemodialysis

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ABSTRACT

Background and aims: Omega-3 (n-3) polyunsaturated fatty acids (PUFAs) are widely recognized to have beneficial effects against cardiovascular disease. We investigated the association of n-3 PUFAs levels with carotid atherosclerosis in patients on hemodialysis (HD), who are at high risk for cardiovascular events.
Methods: Carotid ultra-sound was performed in a total of 461 patients on HD (male 67%, age 67 ± 12years, diabetes rate 46%). Intima-media thickness (IMT) and the plaque score (PS) in carotid arteries were measured. Carotid atherosclerosis was defined as IMT >1.2 mm and/or PS > 5.0. The levels of n-6 PUFAs [dihomo-gamma-linolenic acid (DHLA) and arachidonic acid (AA)] and n-3 PUFAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] were also measured prior to carotid ultra-sound.
Results: Carotid atherosclerosis was observed in 94 patients (20.4%). Individual PUFAs levels were comparable between patients with and without carotid atherosclerosis. However, the ratio of EPA/AA and that of n-3/n-6 PUFAs were significantly lower in patients with carotid atherosclerosis compared to those without (median 0.36 *vs.* 0.41, $p = 0.031$ and 0.85 *vs.* 0.93, $p = 0.041$, respectively]. After adjustment for other confounders, the ratio of EPA/AA (OR 0.30, 95% CI 0.12–0.70, $p = 0.0055$) and the ratio of n-3/n-6 PUFAs (OR 0.45, 95% CI 0.25–0.80, $p = 0.0066$) showed an independent reverse association with carotid atherosclerosis. In addition, the area under receiver-operating characteristic curves for carotid atherosclerosis was significantly greater in an established risk model with EPA/AA and n-3/n-6 ratios than in the established risk model alone.
Conclusions: These data suggest that low ratios of both EPA/AA ratio and n-3/n-6 PUFAs were closely associated with carotid atherosclerosis in patients on HD.

1. Introduction

Omega-3 (n-3) polyunsaturated fatty acid (PUFA) levels can predict cardiovascular disease [1–3]. According to a number of epidemiological studies, consumption of n-3 unsaturated fatty acids, represented in fish oils, and eicosapentaenoic acid (EPA) in particular, is inversely related to the risk of development of coronary artery and cerebrovascular diseases. In addition, the EPA/arachidonic acid (AA) ratio is related to coronary plaque complexity and composition [4,5]. Therefore, many guidelines recommend high intake of fish oil and n-3 unsaturated fatty acids to lower cardiovascular risk [6,7].

At the same time, it is well established that patients on hemodialysis (HD) are at high risk of cardiovascular events [8,9]. In the affected individuals, blood levels of n-3 PUFAs have been reported to be suboptimal as compared to the general population [10]. However, the association between n-3 PUFAs levels and

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atherosclerosis has not been fully evaluated in patients on HD. The present study was conducted with the working hypothesis that low EPA/AA and n-3/n-6 PUFA ratios were related to carotid atherosclerosis in patients on HD.

2. Materials and methods

2.1. Study population

We performed carotid ultra-sound on a total of 461 consecutive outpatients stably undergoing maintenance HD therapy in Nagoya Kyoritsu Hospital and Ama Kyoritsu Clinic. The levels of n-6 PUFAs [dihomo-gamma-linolenic acid (DHLA) and arachidonic acid (AA)] and n-3 PUFAs [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] were also measured prior to carotid ultra-sound. In advance, we excluded patients with cancer and/or active inflammation.

The study conformed to the guidelines of the ethics committee of the institution and was conducted according to the Declaration of Helsinki. The physicians obtained written informed consent from each patient.

In the study, diabetes mellitus was diagnosed if patients had a history of diabetes, a fasting plasma glucose concentration >126 mg/dL, a random plasma glucose concentration of >200 mg/dL and/or HbA1c levels ≥6.5%. Dyslipidemia was defined as medication-dependent or previously known dyslipidemia or low-density lipoprotein cholesterol ≥140 mg/dL or total cholesterol ≥220 mg/dL. Hypertension was defined as medication-dependent, previously known or systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg. Smoking status was defined as positive if patients were smoking currently or had stopped smoking within 6months before checking ultrasonography.

2.2. Measurement of carotid intima-media thickness (IMT) and plaque score (PS)

Ultrasonography of the bilateral common carotid artery (CCA), carotid bifurcation, and internal carotid artery (ICA) was performed using a GE Vivid 7 (GE Vingmed Ultrasound AS, Horten, Norway) ultrasound system with a linear array 3–11 MHz transducer. Intima-media thickness (IMT) was calculated as the mean maximal IMT of the six segments, the near and far walls of the left and right CCA, bifurcation, and ICA. Plaque score (PS) in the carotid arteries were measured in line with an earlier study [11]. Carotid atherosclerosis was defined as IMT >1.2 mm and/or PS > 5.0 [12]. Two independent operators who were blinded to patients' characteristics performed the ultrasonography.

2.3. Measurement of blood samples

Fasting blood samples were obtained from vein on a next dialysis day when ultrasonography was performed. The serum PUFAs composition, including the levels of EPA, DHA, and AA, was measured by gas chromatography at a commercially available laboratory (BML, Tokyo, Japan). The intra- and inter-assay coefficients of variation for the EPA, DHA, DHLA and AA measurements were 2.8% (35.9 μg/mL) and 2.8% (35.3 μg/mL), 0.9% (181.4 μg/mL) and 3.2% (174.2 μg/mL), 2.3% (34.7 μg/mL) and 4.1% (34.3 μg/mL), and 1.7% (168.7 μg/mL) and 3.3% (164.0 μg/mL), respectively.

2.4. Statistical analysis

In the study, statistical analysis was performed using SAS ver. 9.2 software (SAS institute, NC, USA). Variables with a normal distribution were expressed as mean ± SD, and asymmetrically distributed data were presented as median and interquartile range (IQR). Baseline differences of patients' characteristics at baseline between the two groups were evaluated by Student's t-test or Mann-Whitney's *U* test for continuous variables and with the Chi-square test for categorical variables. Significant factors associated with carotid atherosclerosis were evaluated using multivariate Logistic regression analysis including crudely variables with $p < 0.1$ by univariate analysis. In this analysis, each PUFA was also individually evaluated to avoid contamination due to multicollinearity. In addition, we calculated area under receiver-operating characteristic curve (AUC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to assess whether the accuracy of predicting carotid atherosclerosis improved if EPA/AA ratios and n-3/n-6 PUFAs ratios were added to a baseline model with established risk factors, including gender, age, duration of HD, diabetes, hypertension, dyslipidemia, smoking status, body mass index, previous history of cardiovascular diseases, serum albumin, and C-reactive protein. The NRI indicates relatively how many patients improve their predicted probabilities for carotid atherosclerosis, while IDI represents the average improvement in predicted probabilities for carotid atherosclerosis after adding these variables into the baseline model.

In all analyses, $p < 0.05$ was considered statistically significant.

3. Results

The inter- and intra-observer variabilities of IMT measurements defined by Spearman correlation coefficients were 0.84 ($p < 0.0001$) and 0.88 ($p < 0.0001$), respectively. As to PS, they were 0.80 and 0.83, respectively. Table 1 shows the clinical characteristics of the enrolled patients. Of enrolled subjects, 307 (66.6%) were male gender; 210 (45.6%) had diabetes; and 347 (75.3%) had hypertension. The mean age was 67 ± 12 years and the mean duration of HD was 8.9 ± 7.7 years.

Carotid atherosclerosis was seen in 94 patients (20.4%). Although n-3 PUFAs were higher in the group with carotid atherosclerosis compared to that without, there were no significant differences in individual PUFAs between patients with and without carotid atherosclerosis.

Table 2 shows laboratory findings for n-3 and n-6 PUFA levels. The ratio of EPA/AA and that of n-3/n-6 PUFAs were significantly lower in patients with carotid atherosclerosis compared to those without [0.36 (0.21–0.56) *vs.* 0.41 (0.27–1.05), $p = 0.031$ and 0.85 (0.61–1.19) *vs.* 0.93 (0.70–1.28), $p = 0.041$, respectively] (Fig. 1). After adjustment for other confounders, the ratios of both EPA/AA (OR 0.30, 95% CI 0.12–0.70, $p = 0.0055$) and n-3/n-6 PUFA (OR 0.45, 95% CI 0.25–0.80, $p = 0.0066$) were both independently associated with carotid atherosclerosis. Age (OR 1.03, 95% CI 1.00–1.05, $p = 0.032$), diabetes (OR 1.42, 95% CI 1.01–2.01, $p = 0.049$), past history of stroke (OR 2.12, 95% CI 1.09–4.13, $p = 0.028$), and prevalence of peripheral artery disease (OR 2.01, 95% CI 1.10–3.65, $p = 0.023$) were other independent predictors of carotid atherosclerosis (see Tables 3 and 4).

The AUC, NRI and IDI for carotid atherosclerosis were significantly greater when the EPA/AA and n-3/n-6 ratios were added to the established risk model alone (Table 5).

4. Discussion

Cardiovascular disease is the leading cause of death in patients on HD, possibly because they generally have traditional risks factors such as diabetes and hypertension. In the present study, we obtained compelling evidence that low EPA/AA and n-3/n-6 PUFA ratios were closely associated with carotid atherosclerosis in patients on HD.