A Feasbility Study of Erythrocyte Long Chain Omega-3 Polyunsaturated Fatty Acid Content and Mortality Risk in Hemodialysis Patients

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Abstract

Objective—Long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) are increasingly believed to be cardioprotective. We tested the hypothesis that erythrocyte n-3 PUFA levels, as measured by the omega-3 index (O3I), are inversely related to mortality in hemodialysis patients.

Design and Study Population—Retrospective study of 93 prevalent urban U.S. hemodialysis patients with baseline n-3 PUFA blood levels.

Main Outcome Measure—Mortality rate over a median period of 755 days.

Results—The median omega-3 index was 4.69 mean weight %. During the follow up period, 19 patients died and 8 underwent renal transplantation. The probability of survival was significantly greater in patients with an O3I above the median (p=0.025). Univariate analyses found that increasing age and the Charlson Comorbidity Index (CCI) were associated with reduced survival, while a higher O3I and black race were linked with greater survival. In a multivariate model only the CCI score clearly predicted mortality (Hazard Ratio (95% CI); 1.31(1.06, 1.62)), though a protective trend was observed with an O3I above the median (2.48(0.88, 6.95)).

Conclusion—Though this modest sized study did not find a statistically significant relationship between erythrocyte n-3 PUFA levels and mortality, an inverse association was suggested. The existence of such a relationship will need to be confirmed in cohorts with greater statistical power.

INDEX WORDS

dialysis; mortality; omega-3; fish oil; eicosapentaenoic; docosahexaenoic

INTRODUCTION

The long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acids (DHA, 22:6n-3) are important biological mediators obtained principally from dietary marine sources. A growing body of laboratory and clinical data suggest that n-3 PUFA have cardioprotective benefits and reduce mortality, perhaps through their anti-inflammatory, anti-arrhythmic, lipid-lowering, and/or anti-hypertensive effects (1). Based on this evidence the American Heart Association (AHA) recently recommended that individuals at high cardiovascular risk consume 1 g daily of fish oil (a rich source of EPA and DHA) (1), since supplementation rapidly and effectively raises blood n-3 PUFA levels (2).

We have previously demonstrated that hemodialysis patients consume amounts of dietary fish far below AHA guidelines and consequently have suboptimal blood n-3 PUFA content (2,3). Inadequate n-3 PUFA intake may potentiate the already heightened cardiovascular risk state seen with kidney failure. Though an inverse link between fish consumption and mortality in dialysis patients has been reported (4), there are no data examining the relationship between blood n-3 PUFA content and death. Such data would help confirm that n-3 PUFA, and not fish, are the true mediators of cardioprotection.

We tested the hypothesis that erythrocyte n-3 PUFA levels, a reflection of long-term consumption, are inversely related to risk of death in a cohort of hemodialysis patients.

MATERIALS AND METHODS

Study Population and Design

Ninety three prevalent hemodialysis patients from Indiana University (Indianapolis, IN) affiliated dialysis units underwent blood draws between March 2005 and April 2006. A detailed description of the study cohort has been provided elsewhere (2,3,5). Briefly, the subjects were free of acute illness, had blood omega-3 levels measured at baseline, were followed until time of death, and adhered to the following exclusion criteria: age less than 18 years; fish oil or omega-3 supplementation within the past six months; pregnancy; current enrollment in a dietary study; compliance with a diet that prohibits fish intake; inability to give written informed consent; and malabsorption syndromes such as chronic pancreatitis or chronic diarrheal illnesses. The relevant institutional review boards approved the study protocol, and all patients gave written informed consent after reviewing a written summary of the study plan. The study adhered to the Declaration of Helsinki.

Measurements and Laboratory Tests

Blood samples were obtained immediately prior to hemodialysis treatment while in the fasting state and immediately placed on ice. Plasma and whole red blood cells (RBC) were separated by centrifugation within the hour, washed several times with 0.9% saline, and stored at $-80°$ C. Lipids from RBC were extracted with chloroform/methanol (2:1, v/v) and fatty acid methyl esters (FAME) prepared by derivatization using boron trifluoride (BF_3) in methanol (10% w/ w, Supelco Inc. Bellefonte, PA, U.S.A.) as previously described (6). FAME were extracted with isooctane and analyzed by a gas chromatograph (Agilent 6890 Plus, autosampler 7683, Chemstation Rev. A.08.03; Agilent Technologies, Inc., Wilmington, DE, U.S.A.) equipped with a flame ionization detector and a DB-23 fused silica capillary column (30 m, 0.53 mm i.d., 0.5 µm film thickness; Agilent) using helium as the carrier gas. FAME were identified by comparison of their retention times with authentic standards (Nu-Chek-Prep, Inc., Elysian, MN, U.S.A.) and fatty acid values expressed as weight percentages. The summation of

erythrocyte EPA and DHA content, the omega-3 index, has been linked to clinical outcomes and was used in this study as a marker of blood n-3 PUFA content (7).

All subjects completed a demographic, social, and fish consumption questionnaire. The Charlson Comorbidity Index (CCI), an index of overall disease burden that has been validated to predict mortality, was estimated for each patient (8). Serum albumin was measured by standard laboratory technique.

Statistical Analysis

Variables are expressed as mean ± SD or frequency. Study entry characteristics were compared between patients with omega-3 index above the median and below the median by use of a Student's t-test, Wilcoxon Rank Sum test or chi-square. The primary outcome was time to death. Kaplan-Meier survival curves were compared between the two groups using log-rank test. Transplanted patients were censored at time of transplantation. The relative risk of death was estimated for individual covariates with a Cox proportional-hazards model. Sex, race, fish servings, age, serum albumin, body mass index and Charlson Comorbidity Index were considered for possible confounding factors. Variables significantly associated with outcome at the univariate Cox regression analysis (i.e. omega-3 index, Charlson Comorbidity Index, age, race) were entered in a multivariate model and adjusted omega-3 index effect was assessed based on the likelihood-ratio test. All analyses used two-sided P values with significance if $\leq$ 0.05. Data were analyzed with SAS 9.3.1 (SAS Institute, Inc., Cary, NC).

RESULTS

Forty seven and 46 patients had levels below and above the median omega-3 index of 4.69 mean weight %, respectively. As seen in Table 1, the latter group included more blacks and consumed more dietary fish.

Over a median follow up period of 755 days (range: 40–770), 19 patients died and 8 underwent renal transplantation. Erythrocyte omega-3 index levels (median (range)) in persons who died and survived were 4.9 (1.3–8.1) and 4.3 (1.6–6.8) mean weight %, respectively. As shown in the Figure, the unadjusted probability of survival was significantly greater in patients with omega-3 index above the median (p = 0.025).

Univariate analyses found that an increasing age [HR (95% CI); 1.05 (1.01, 1.09)] and CCI score [1.47 (1.2, 1.8)] were associated with reduced survival, while a higher omega-3 index (above vs. below median, [0.35 (0.13, 0.92)]), and black race [vs. white; 0.25 (0.10, 0.62)] were linked to greater survival. When examined as a continous variable, an increasing omega-3 index was associated with improved survival [0.64 (0.44, 0.94)]. A multivariate Cox model (Table 2) found that only the CCI score clearly predicted mortality, though a protective trend was observed in persons with an omega-3 index above the median.

DISCUSSION

Our study is the first to directly examine the relationship in hemodialysis patients between blood n-3 PUFA content and mortality. Though the findings were not statistically significant, they did suggest an inverse association, especially considering the cohort's modest size and limited statistical power. Identifying a link between blood n-3 PUFA levels and risk of clinical events in the kidney failure population is a worthwhile goal simply because, as we have recently demonstrated (2), levels are easily modifiable by oral supplementation.

N-3 PUFA play important and increasingly recognized roles in a number of biological processes that influence cardiovascular health, including eicosanoid production, regulation of

the inflammatory and immune systems, lipid metabolism and blood pressure, and cell membrane function and stability (9–12). There is robust observational evidence (13–15) and mounting data from clinical supplementation trials (16–18) that n-3 PUFA reduce the risk of cardiovascular events, sudden cardiac death, and overall mortality. The evidence is persuasive enough to have convinced the AHA and other international health organizations to establish n-3 PUFA intake guidelines for at-risk individuals (1,19).

The cardiovascular effects of n-3 PUFAs have not been extensively explored in the dialysis population, though the available evidence does suggest a benefit. Kutner et al observed that fish consumption was associated with reduced mortality in a retrospective analysis of 216 incident dialysis patients (4). N-3 PUFA were not measured in this study. A more recent, randomized, secondary prevention study in Danish hemodialysis patients found that fish oil supplementation 1.7 g/day reduced the risk of myocardial infarctions but not the primary composite outcomes of total deaths and cardiovascular events (20). Though well designed overall, the study was underpowered to detect the primary outcome.

In conclusion, though our modest sized study did not find a clear, statistically significant link between n-3 PUFA blood levels and mortality, it did suggest an inverse relationship. The existence of such a relationship will need to be confirmed in cohorts with greater statistical power.

ACKNOWLEDGMENTS

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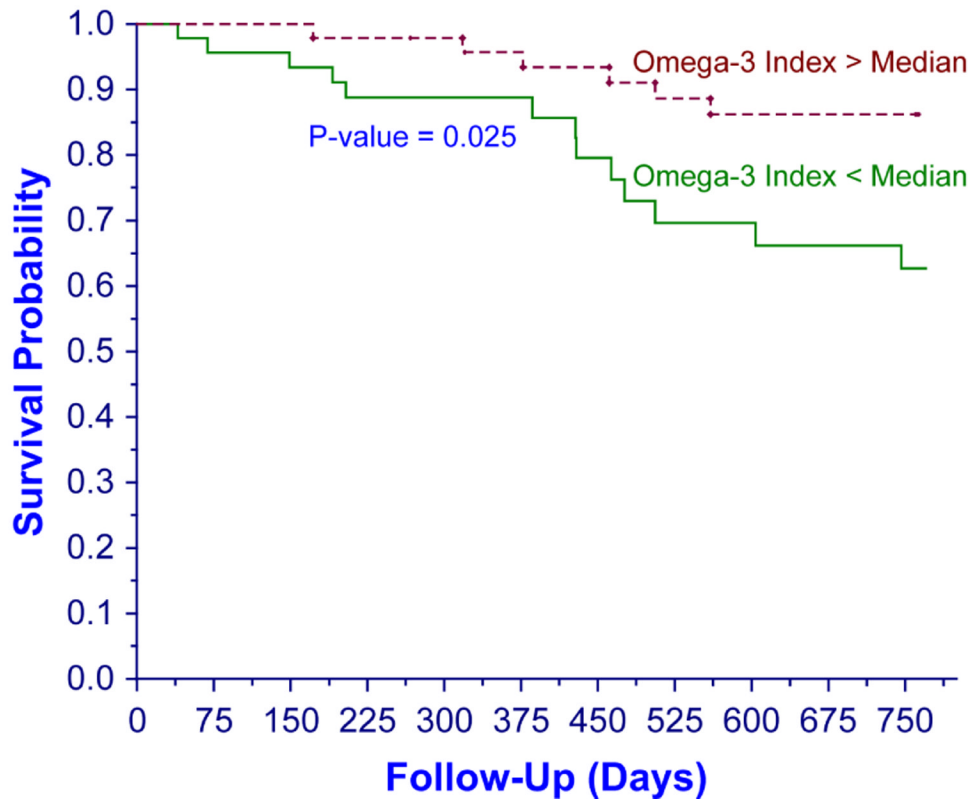
Figure.
Survival by Omega-3 Index

Table 1

Study Subject Characteristics

Characteristics	Omega-3 Index		P
	Below Median (n=47)	Above Median (n=46)	
Age [*]	53 ± 13	56 ± 12	0.24
Sex (% male)	72.3	58.7	0.17
Race (%)			<0.01
White	36.2	10.9	
Black	63.8	89.1	
Body Mass Index (kg/m2) [*]	29 ± 8	31 ± 9	0.11
Serum Albumin (g/dl) [*]	3.7 ± 0.4	3.7 ± 0.4	0.82
Diabetes Mellitus (%)	19	13	0.43
Peripheral Vascular Disease (%)	34	24	0.29
Myocardial Infarction (%)	19	26	0.43
Charlson Comorbidity Index [*]	4.9 ± 2.0	4.4 ± 2.0	0.22
Weekly Fish Servings (%)			<0.01
<1	59.1	23.9	
≥1	40.9	76.1	

[*] mean ± SD

Table 2

Hazard of Death in a Multivariate Model

Variable	Hazard Ratio[*] (95% CI)	P value
Omega-3 Index (<median)	2.48 (0.88, 6.95)	0.085
Charlson Comorbidity Index Score	1.31 (1.06, 1.62)	0.011
Age	1.03 (0.99, 1.07)	0.118
Black (versus white)	0.50 (0.18, 1.37)	0.174

[*] Hazard ratio was per one unit increase for continuous variables