ORIGINAL RESEARCH

Development of Kidney Transplant Fibrosis Is Inversely Associated With Plasma Marine Fatty Acid Level

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Objective(s): We assessed associations between plasma levels of polyunsaturated fatty acids (PUFAs) and degree of inflammation and interstitial fibrosis in transplanted kidneys.

Design: The design of the study was single center cohort study.

Subjects: A study population of 156 patients who received a kidney transplant at Oslo University Hospital during 2010.

Main Outcome Measure: Kidney transplant biopsies were obtained at 2 months and 1 year after transplantation. Degree of inflammation and interstitial fibrosis in the cortex of transplanted kidneys were estimated semi-quantitatively. Plasma phospholipid fatty acids levels were measured in a stable phase 2 months posttransplant. We used multivariate linear regression to assess associations between plasma levels of PUFAs and degree of inflammation and interstitial fibrosis at 2 months and 1 year postoperatively and change in degree of interstitial fibrosis during the first year after transplantation, adjusting for inflammation and fibrosis risk factors.

Results: Higher plasma marine n-3 PUFA levels were associated with less development of interstitial fibrosis in the kidney transplant (unstandardized β-coefficient −1.12, standardized β-coefficient −0.18, $P = .03$) during the first year after transplantation. Plasma levels of alpha linoleic acid, linoleic acid, and arachidonic acid were not associated with development of interstitial fibrosis. No associations were found between plasma levels of PUFAs and inflammation inside fibrotic areas or outside fibrotic areas in the kidney transplant at neither 2 months nor 1 year postoperatively. Linolenic acid levels in plasma were positively associated with change in renal function during the first year after transplantation.

Conclusion: The inverse association between plasma marine n-3 PUFA levels and development of interstitial fibrosis during the first year after kidney transplantation suggests that marine fatty acid consumption might halt progression of fibrosis.

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Study population: Adult kidney transplant recipients. Single center cohort (Oslo University Hospital, Rikshospitalet).

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Introduction

DESPITE IMPROVED SHORT-TERM survival of transplanted kidneys during the last decades, long-term transplant survival has remained virtually unchanged.[1] Injury to the donor organ before transplant surgery and ischemia–reperfusion injury related to the transplant procedure often dominate in early protocol biopsies, while silent interstitial cell infiltration, alloimmune, and nonimmunologic tissue injury may contribute to inflammation and fibrosis in kidney transplants beyond the early postoperative phase.[2-4] Fibrosis is a key histologic correlate for progression of chronic allograft nephropathy as well as chronic kidney disease in native kidneys.[5] Inflammation may be linked to development of fibrosis in transplanted organs, partly by release of profibrotic cytokines and activation of fibroblasts.[6,7] Nutritional factors that may influence inflammatory and fibrotic mechanisms are sought, including the essential polyunsaturated fatty acids (PUFAs). Marine n-3 PUFA could moderate the cascade from inflammation to fibrosis through several mechanisms, including competitive inhibition of the marine n-3 PUFA eicosapentaenoic acid (EPA) on the n-6 PUFA arachidonic acid (AA) as substrate in the synthesis of prostaglandins and leukotrienes.[8] Interestingly, experimental studies data suggest that marine n-3 PUFA may also have direct antifibrotic effects, including reduced fibroblast transformation and proliferation, reduced collagen synthesis, and reduced transition from endothelial or epithelial to mesenchymal cells.[9,10] In humans, a recent trial of myocardial infarction survivors found a lower degree of interstitial fibrosis outside infarct scars in patients on high-dose marine n-3 PUFA supplementation.[11] Less is known about effects on inflammation and fibrosis from consumption of the plant-derived n-3 PUFA alpha linolenic acid (ALA) and the major n-6 PUFAs linoleic acid (LA) and AA although consumption of ALA and LA has been associated with less inflammation and less glycemia in obesity and diabetes.[12,13] To the best of our knowledge, no previous study has evaluated PUFAs in relation to histopathologic features in kidneys. The objective of this study was to assess whether plasma levels of PUFAs were associated with development of interstitial fibrosis during the first year after transplantation and degree of inflammation inside and outside of fibrotic areas.

Materials and Methods

Study Design and Clinical Data Collection

The study population has previously been described.[14,15] In this single center study, 156 of 254 adult patients who received a kidney transplant during the year of 2010 were included. They had an ABO compatible donor and adequate kidney transplant core needle protocol biopsies performed at 2 months and 1 year postoperatively. Fifteen patients had missing data on plasma fatty acid levels.

Clinical data were retrieved from medical charts. Blood was sampled at a clinical visit 10 weeks after kidney transplantation. Routine clinical chemistry analyses were performed in fresh fasting blood samples. For this study, aliquots for determination of plasma fatty acid composition were immediately frozen and stored at −80°C for 2 to 3 years before they were sent to The Lipid Research Center, Aalborg University Hospital, Denmark, for analysis. In short, individual plasma phospholipid fatty acid levels were identified and quantitated as weight percentage (wt%) of total plasma phospholipid fatty acids by gas chromatography.[14] Plasma marine n-3 PUFA levels were defined as the sum of EPA, docosahexaenoic acid, and docosapentaenoic acid.

The standard immunosuppressive protocol, infectious disease monitoring, and histocompatibility testing have previously been described in detail.[15] In immunologically standard risk patients, the immunosuppressive protocol consisted of induction therapy with basiliximab, followed by maintenance therapy with prednisolone, mycophenolate, and a calcineurin inhibitor (either cyclosporine A or tacrolimus). The choice of calcineurin inhibitor was based on recipient age, diabetes mellitus, and body mass index in most cases, thus no meaningful comparison of the 2 calcineurin inhibitors can be made in this cohort. Four patients were diagnosed with polyomavirus-associated nephropathy and 23% of patients received antiviral therapy either preemptive or as treatment of cytomegalovirus infections during the first year after kidney transplantation. Biopsy-proven acute rejection episodes were experienced by 20% of the patients during the first year after engraftment. Acute rejection episodes were treated with intravenous methylprednisolone followed by a transiently increased dose of oral prednisolone. Half of the patients (52%) received statin therapy at the time of the 1-year clinical visit.

Histologic Data Collection

Kidney transplant biopsies sampled at 6 ± 2 weeks and 1 year posttransplant ± 2 weeks were fixed in 4% formalin, embedded in paraffin, and stained with hematoxylin–eosin–saffron, periodic acid–Schiff, and trichrome. Light microscopy with semi-quantitative estimation of histopathologic features in the cortex of kidney transplants was determined by 2 experienced pathologists (H.S. and F.P.R.), who were blinded to the identity of the patient and clinical information. Percent inflammation in the renal cortex was determined inside and outside of fibrotic areas. The degree of interstitial fibrosis in the renal cortex was determined on a 10% scale (0-9) and percent interstitial fibrosis was defined as the central value of each step in the 10% scale (5%, 15%, and so on up to 95%). This approach should improve resolution in fibrosis grading compared with the 0-3 scaled Banff classification and is considered nearly as reliable as morphometry.[15-18]