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INFLAMMATION, NUTRITIONAL STATUS, PUFA PROFILE AND OUTCOME IN HEMODIALYSIS PATIENTS

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Abstract - Patients with end-stage renal disease (ESRD) include a significant percentage of malnourished patients with other risk factors: dyslipoproteinemia, insulin resistance, increased oxidative stress and inflammation that together impair endothelial function. Abnormal polyunsaturated fatty acids (PUFA) patterns are reported in patients with ESRD. The basic mechanisms of these disorders are connected with changes in cell functions at the membrane level. Vascular smooth muscle cell proliferation plays an important role in the pathogenesis of atherosclerosis. We have examined the association between atherosclerotic risk factors and nutritional status in hemodialysis (HD) patients. Mortality was followed for up to 18 month. Forty-three HD patients were examined (20 males, 23 females, ages 55±12 years). Nutritional and inflammatory markers, including serum concentrations of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-alpha), and interleukin 6 (IL-6), were measured. There was significant positive correlation between the plasma albumin level and CRP. Significant correlation was found between plasma the cholesterol level and some PUFA. Increasing inflammation and endothelial dysfunction predict the development of vascular disease. We report on the relationship between inflammatory markers and nutritional parameter, indices of atherosclerosis and other cardiovascular risk factors in patients on hemodialysis.

Key words: Malnutrition, inflammation, hemodialysis, PUFAs

INTRODUCTION

Malnutrition and inflammation are associated with end-stage renal disease (ESRD). IL-6 and TNF-alpha powerfully predict death from cardiovascular disease in dialysis patients as well as the progression of vascular injury. Atherosclerosis and inflammation have similar basic mechanisms involving the adhesion of leukocytes to the vascular endothelium in their early phases. Major predictors of the clinical outcome in dialysis patients are protein-energy malnutrition (PEM) and inflammation. A common mechanism for the development of cardiovascular disease and malnutrition in dialysis patients can be cytokine activation (Stenvinkel et al., 2001; Zimmermann et al., 1999; Bologa et al., 1998; Kaizu et al., 1998).

Inflammatory states in end stage renal disease (ESRD) are associated with an elevation of serum acute phase proteins, including CRP and some pro-inflammatory cytokines such as IL-6 and TNF alpha (Stenvinkel et al., 2001;Ross, 1999). IL-6 is reported to play a central role in the pathophysiology of the adverse effects of inflammation in ESRD patients. The increased activation of inflammatory

cytokines such as IL-6 and TNF-alpha, may cause muscle breakdown and hypoalbuminemia, and may be involved in atherogenesis (Ebisuri et al., 1995; Tsujinaka et al., 1996; King et al., 1998; Stenvinkel et al., 1999).

Dyslipidemia is one of the possible risk factors for advanced atherosclerosis in patients with chronic renal failure. Abnormal phospholipid metabolism may play an important role in the progression of atherosclerosis in patients with renal failure. The total concentration of serum non-esterified fatty acids (NEFA) plays an important role in the pathogenesis of cardiovascular disease (CVD). Individual circulating NEFA have achieved less scientific focus in relation to atherosclerosis compared to total NEFA. These basic discoveries help us in understanding the damage process of the endothelium and the appearance of its dysfunction by the inflammation and lipid peroxidation processes. A strong link exists between chronic inflammation and nutritional markers in HD patients (Li et al., 2003; Lowrie et al., 1990). The biological effects of n-3 fatty acids have been shown in studies of animals and humans in which anti-inflammatory and antithrombotic effects could be of relevance in HD patients. It is widely accepted that a dietary intake of n-3 PUFA or fish oil as their dietary sources has beneficial health effects on sick and healthy individuals. A great number of clinical studies have shown that the intake of n-3 fatty acids reduces the incidence of cardiovascular events and plays an important role in the prevention of inflammatory and autoimmune disorders (Simopoulos et al., 1991; Burr et al., 1989).

HD patients have an impaired essential fatty acid status, as well as a high level of oxidative stress, and they are very often malnourished with a high risk for developing cardiovascular pathology. The primary aim of our study was to establish the association between markers of inflammation and atherosclerosis and the parameters of malnutrition in HD patients. The secondary aim of this study was to determine the mortality rate, which was followed for up to 18 month.

MATERIALS AND METHODS

We examined 43 HD patients (mean age 55±12 years, mean dialysis duration 47±8 months) who were prospectively followed-up for 18 months and all cause and cardiovascular mortality were registered. The patients had been treated with hemodialysis three times a week for at least 6 months. Patients with diabetes, acute infections and malignancies were excluded from the study. The duration of dialysis was defined as the number of months from the initiation of chronic hemodialysis to the time of laboratory data collection. All blood samples were obtained immediately before the dialysis session after an 8-hour fast on a mid-week dialysis session. Plasma levels of albumin, creatinine, hemoglobin, IL-6, TNF-α, high sensitivity CRP, total cholesterol, triglyceride, HDL-cholesterol, LDL cholesterol and PUFA content of red cell membrane phospholipids were measured at the start of the study. Plasma albumin, creatinine and lipids were measured by routine laboratory methods. Plasma CRP level, TNF-α and IL-6 were measured by enzyme-linked immunosorbent assay kits (ELISA). The total fatty acids composition was determined by gas chromatography (GS) in the methyl esters form. GS analysis was performed on a VARIAN chromatographer, Model 1400.

The body mass index (BMI) was recorded. Lean body mass (LBM) was measured by bioelectric impedance (BIA). We used standard Doppler echo examinations to determine plaques on the carotid arteries.

The University Clinical Research Ethics Committee approved the study. All 43 hemodialysis patients recruited from our unit gave written informed consent.

Statistical analysis

All statistical analysis was performed with Stat 6.0 software package on MS Windows (Stat soft Inc., Novi Sad Serbia). The t-test and Mann-Whitney test were applied for statistical comparisons between groups. Correlations were tested with regression

Table 1. Comparison of investigated parameters between groups according BMI tertiles

Variables	1st tertile (group I)	3st tertile (group II)
Number	19	23
Albumin g/L	31±3.01*	33±3.21
TNF-α pg/ml	2.08±1.65	1.95±1.53
IL-6 pg/ml	4.50±4.19*	3.41±3.39
Carotid plaques %	62*	39
CVD %	89*	72

*P should be presented as < 0.05

Table 2. Comparison of inflammatory markers between groups

	1st tertile (group I)	3st tertile (group II)
age	49±11	51.8±12
CRP(mg/L)	1.42±8.53	7.89±10.54*
IL-6(pg/ml)	1.16±3.63	4.25±3.85*
TNF-α(pg/ml)	0.84±1.21	2.83±1.58*

*P should be presented as < 0.05

Table 3. Pearson's partial coefficient correlation:

	Cholesterol	p-level
Hb	0.27512	0.240
Creat.	-0.38435	0.094
TG	0.89735	0.000
CRP	0.16543	0.485
Album.	-0.3989	0.081
TNF-α	-0.06838	0.774
IL-6	0.23925	0.309
HDL-cholesterol	0.42546	0.061
LDL-cholesterol	0.98452	0.000
20:2(n-6)	-0.58545	0.006
20:3(n-6)	0.52257	0.018
20:4(n-6)	0.1855	0.433
20:5(n-3)	-0.69022	0.000
22:5(n-3)	-0.48607	0.029
22:5(n-3)	0.12718	0.593
22:6(n-3)	0.14631	0.538

Table 4. Correlation matrix:

Variables	CRP	albumin	age	Duration HD	creatinine	trigliceride	cholesterol	HDL-chol.	LDL-chol.	BMI	plaques
CRP	-	-0.4897**	0.2511	0.1679	-0.0657	-0.0807	0.0727	-0.0501	0.1088	0.0497	0.2952
albumin		-	0.0132	0.0363	0.0363	0.2107	-0.0303	-0.0737	0.0548	0.1038	-0.0390
age			-	-0.0211	-0.3403*	0.2316	0.1372	-0.0733	0.0646	0.2483	0.4242**
Duration HD				-	-0.0072	0.1067	-0.1848	-0.4411**	0.0262	-0.1413	0.1924
creatinine					-	-0.1934	-0.1476	0.0762	-0.1374	0.1696	-0.1876
trigliceride						-	0.5447**	-0.5171**	0.5293**	-0.0009	0.3424*
cholesterol							-	-0.0995	0.7990**	-0.0750	0.2357
HDL-chol.								-	-0.2934	-0.0228	-0.3218*
LDL-chol.									-	-0.1174	0.1931
BMI										-	0.0635
plaques											-

* Significant at the level of 5%
** Significant at the level of 1%

analysis and Pearson's partial correlation. Mortality was analyzed with Kaplan-Meier curves according CRP tertiles values.

RESULTS

The group of well nourished of HD patients with higher levels of lean body mass had better nutritional parameters and lower levels of inflammatory parameters (Table 1).These patients presented BMI and other nutritional parameters significantly higher than patients with lower levels of LBM. Malnourished patients had significantly increased cardiovascular disease and carotid plaques.

Inflammatory markers (CRP, IL-6 and TNF-α) were higher in malnourished patients and these differences were significant compared to well-nourished group (Table 2).

The plasma cholesterol value correlated with some PUFAs (Table 3). There were negative correlations with fatty acid, namely 20:2(n-6) and fatty acid, namely 20:5(n-3) and 22:5(n-3).

There was correlation between the plasma CRP and albumin value. There were strong positive correlations between LDL-cholesterol and other lipids (cholesterol and triglyceride). HDL-cholesterol negatively correlated with dialysis duration and triglycerides. The presence of plaques positively correlated with age and triglyceride level and negatively correlated with HDL-cholesterol level (Table 4).

A high plasma level of CRP (>5mg/dL) indicated an increased risk of mortality. The highest risks of mortality were reached in patients with high CRP plasma values in the III tertiles (Fig. 1).

DISCUSSION

This cross-sectional study confirmed an atherosclerotic risk in a high percentage of HD patients. The characteristics of these patients, such as elevated plasma lipids, nutritional parameters (dry weight, lean body mass and BMI), inflammation and vascular disease (presented as macroangiopathy), were found to be significant predictors of mortality and comorbidity. Many studies have examined inflam-

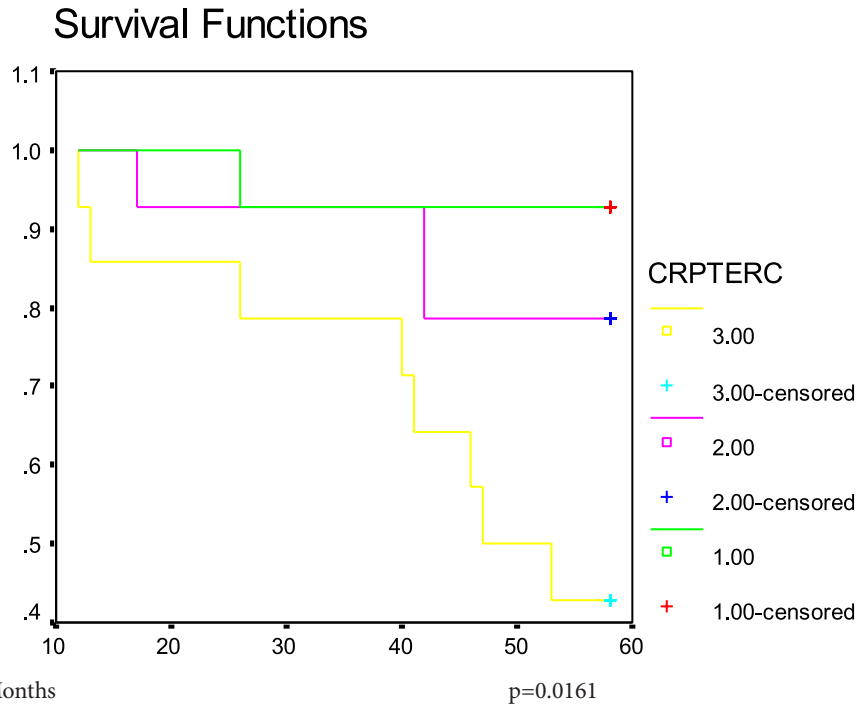

Fig. 1. Cumulative survival and CRP values. Cumulative survival curves estimated from the Kaplan-Meier model according to CRP tertiles.

matory markers in the process of atherosclerosis. The initiation of the inflammatory process in ESRD can be associated with an elevation of serum acute-phase proteins, including CRP and some pro-inflammatory cytokines such as IL-6 and TNF-α. IL-6 is reported to play a central role in the pathophysiology of the adverse effects of inflammation in ESRD patients. Increased activation of inflammatory cytokines, such as IL-6 and TNF-α, may cause muscle breakdown and hypoalbuminemia and may be involved in atherogenesis (Stenvinkel et al., 2001; Ross, 1999; Ebisuri et al., 1995; Stenvinkel et al., 1999). The risk of metabolic and cardiovascular disorder was greater when the serum albumin concentration was lower.

It was also evident that most of the classical risk factors associated with a tendency to atherosclerotic disease in the general population also play a role in HD patients. Two of these factors, malnutrition and inflammation, are also identified in many clinical studies. It is well recognized that malnutrition and hypoalbuminemia are important predictive factors of mortality in patients with ESRD. In addition, high concentrations of acute-phase proteins (CRP) are strongly associated with death within 1 year in pre-dialysis and dialysis patients. In our study, there was also an association between the CRP and albumin level. Fluid overload and congestive heart failure may be other contributors to increased inflammatory responses in HD patients (Li et al., 2003; Lowrie et al., 1990).

It is now known that chronic inflammation increases the risk of premature atherosclerosis and cardiovascular disease in patients with chronic kidney disease. Correction of the nutritional status in HD patients improves the inflammatory state and decreases the plasma concentrations of inflammatory markers and other parameters that contribute to cardiovascular disease.

Malnourished dialysis patients more often have cardiovascular disease, carotid plaques and elevated

inflammatory cytokines, which all may increase the risk of atherosclerotic vascular disease. These data suggested that the inflammatory markers in malnourished HD patients identify patients at high risk of comorbidity and mortality. The degree of inflammation correlates with disease severity.

Inflammation was associated with indices of malnutrition and an atherogenic lipid, and may contribute to the high frequency of cardiovascular disease in HD patients. End stage renal disease leads to a reduction in lean body mass, which contributes negatively to the outcome in the HD population.

In this study, we have shown that HD patients had reduced PUFAs in the red cell phospholipids. This study has demonstrated the high prevalence of malnutrition, inflammation, carotid plaques and cardiovascular disease. Malnourished dialysis patients more often have cardiovascular disease, carotid plaques and elevated inflammatory cytokines, all of which may increase the risk of atherosclerotic vascular disease.

In our previous study, we observed a reduced content of essential fatty acids in the red cell membrane phospholipids. In the present study, two significant findings were observed: reduced levels of some nutritional parameters (indicative of malnutrition) and increased plasma inflammatory markers (IL-6 and TNF-a). These data indicate that PUFA deficiency may be associated with malnutrition and inflammation in these patients.

Phospholipids play a central role in membrane function. The length and degree of unsaturated fatty acids are determinants of enzyme activity. The activation of peroxisome proliferator-activated receptors might be a possible mechanism for the downregulation of inflammatory markers with supplementation of n-3 fatty acids.

These data indicate that PUFA deficiency may be associated with increased inflammatory cytokines, and their levels after treatment may reflect on better clinical outcome. The red cell membrane is an example of plasma membrane. It is very similar to the vascular endothelium. A decrease of the PUFA content in membrane phospholipids reduces liquidity and the mechanical properties of cell membranes. Such a defect in the endothelial membrane could cause vascular dysfunctions as has been demonstrated in rats. This suggests that the pathology of the inflammatory process is associated with the incorporation of EPA and DHA into membrane phospholipids (Sellmayer et al., 1995; Koorts et al., 2002).

The importance of lipid level as a risk factor in HD patients is more complex and depends on other factors, such as nutritional parameters and inflammatory processes. IL-6 is a central mediator of cardiovascular risks associated with many diverse conditions (Perunicic et al,, 2008; Perunicic et al., 2007).

Peck suggests that HD patients may have increased prostaglandin E2 (PGE2) values and fatty acid profiles indicative of essential fatty acids deficiency (Peck et al., 2007). Sinziger and Leitner have shown that the metabolism of arachidonic acid shifted from the cyclooxygenase pathway to the lipoxygenase pathway in renal patients. Higher level of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) block the lipoxygenase pathway of eicosanoid production, pushing the arachidonic acid metabolism to the cyclooxygenase pathway. This pathway leads to an increased level of prostaglandin E2 (PGE2) that acts less inflammatorily than leukotriene B4 (Peck et al., 1997; Sinzinger et al., 1987).

Our study has demonstrated the high prevalence of malnutrition, inflammation, carotid plaques and cardiovascular disease in HD patients. The results suggest that inflammation is an important process in promoting atherosclerosis. The present study showed that a higher CRP level was significantly associated with a higher risk of mortality in malnourished hemodialysis patients. We calculated the mortality risk based on the one-point measurements of CRP at the start of observation, and not on averaged values during the follow up. This is a limitation of the study.

CONCLUSION

Malnutrition is of greater prognostic importance in the survival of HD patients. However, serum albumin is influenced by factors other than malnutrition, and high CRP is correlated with low serum albumin in malnourished dialysis patients. The observations from this study may offer a basis for future research on new nutritional interventions based on PUFA in delaying the progress of inflammatory process and atherosclerosis in HD patients. The present study indicates that higher as opposed to lower CRP levels are associated with poor prognosis in HD patients. The inflammatory cytokines are aggravated patients with an altered nutritional status.

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