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CLINICAL STUDY

Plasma and Erythrocyte Phospholipid Fatty Acids Composition in Serbian Hemodialyzed Patients

Vanja Ristić, Ph.D., Vesna Tepšić, M.D., M.Sc., and Danijela Ristić-Medić, M.D., M.Sc.
Institute for Medical Research, Laboratory for Nutrition and Metabolism, Belgrade, Serbia and Montenegro

Gordana Peruničić, M.D., Ph.D. and Zorica Rašić, M.D., Ph.D.
Department of Nephrology, University Hospital Zemun, Belgrade, Serbia and Montenegro

Marija Poštić, B.Sc., Aleksandra Arsić, B.Sc., Vera Blaženčić-Mladenović, M.D., M.Sc., and Gordana Ristić, Ph.D.
Institute for Medical Research, Laboratory for Nutrition and Metabolism, Belgrade, Serbia and Montenegro

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 aim of this study was to determine specific characteristics of plasma and erythrocyte phospholipid content and fatty acid composition in 37 patients with chronic renal failure on hemodialysis (HD). The results were compared with the characteristics of healthy subjects. Briefly, plasma triglyceride ($p < 0.001$), total cholesterol ($p < 0.05$), and total phospholipids ($p < 0.01$) levels were significantly higher and HDL-cholesterol level significantly lower ($p < 0.01$) in HD patients. Plasma phosphatidylcholine and phosphatidylethanolamine concentration were significantly higher ($p < 0.001$) in HD patients. The plasma phospholipid fatty acids composition indicated significantly ($p < 0.01$) higher level of oleic (18:1 n-9) and lower levels of eicopentaenoic (20:5 n-3 EPA) and docosahexaenoic (22:6 n-3 DHA) acids ($p < 0.05$). However, in HD patients, the relative concentration of plasma phospholipid n-6 polyunsaturated fatty acid (PUFA) was significantly lower ($p < 0.05$). The fatty acid composition of erythrocyte phospholipid in HD patients was modified with EPA and DHA levels significantly lowered ($p < 0.05$). Our results demonstrate an abnormal phospholipid metabolism and deficiency of n-3 PUFA in plasma and erythrocyte phospholipids in hemodialyzed patients.

INTRODUCTION

Several abnormalities of lipid metabolism occur in patients with chronic renal failure.[1,2] In these patients, dyslipidemia is a characteristic feature and has been implicated in the pathogenesis of cardiovascular complications.[3,4] Compared with the general population, those with end-stage renal disease have a substantially elevated risk of death from cardiovascular disease. Statistics show that 50–60% of all deaths among dialysis patients are secondary to cardiovascular complication.[5] The most common plasma lipid abnormalities in patients with chronic renal failure on hemodialysis are high triglycerides, small LDL particles, and low HDL-cholesterol.[6,7] The lipoprotein abnormalities and hemodialysis appear to be the result of impaired lipoprotein catabolism. A number of investigations have shown that the activities of lipoprotein lipase, hepatic triglyceride lipase, and lecithin cholesterol acyltransferase (LCAT) are reduced in chronic renal failure.[2] LCAT deficiency is common in uremia and is associated with changes not just in plasma lipids but also in membrane lipids, which may be relevant to the progression of chronic renal disease.[8]

Address correspondence to Danijela Ristić-Medić, M.D., M.Sc., Institute for Medical Research, Laboratory for Nutrition and Metabolism, 11 129 Belgrade, P.O. Box 102, Serbia and Montenegro; Fax: +381 11 643691; E-mail: danijelar@imi.bg.ac.yu gordanar2000@yahoo.com

Phospholipids play an essential role in membrane structure and function. The length and degree of unsaturated membrane phospholipids fatty acids are main determinants of fluidity, transport systems, activity of membrane-bound enzymes, and susceptibility to lipid peroxidation.[9–11] The fatty acid profile of serum lipid, especially the phospholipids, reflects the fatty acid composition of cell membranes.[12] There are limited and inconclusive data, which deal with changes in fatty acid metabolism and in fatty acid composition of plasma phospholipids in chronic renal failure.[13–15] Erythrocytes can alter phospholipids fatty acids composition only by the exchange of intact molecules with plasma lipids but cannot alter fatty acid chain length or the degree of unsaturation. It has been established that erythrocytes reflect the general fatty acid metabolism in other organs and tissues.[16,17] The aim of this study was to determine specific characteristics of plasma and erythrocyte phospholipids content and fatty acid composition in patients with chronic renal failure on hemodialysis.

METHODS

Patients

The study was carried out in 37 chronic renal failure patients from the Department of Nephrology, University Hospital Zemun, Belgrade. All patients (21 male, 16 female, mean age 52 ± 10 years, range 42–64) had been on maintenance hemodialysis three times a week. The patients were clinically stable, with adequate nutrition/inflammatory status and with no recorded cardiovascular events (coronary heart disease and cerebrovascular disease). The mean body mass index (BMI) calculated from dry body weight was 23.65 ± 3.52 kg/m^2 (range 19.8–27.3). Serum albumin concentration was 41.1 ± 2.9g/L and serum C-reactive protein level was 3.08 ± 1.48 mg/mL. Patients' characteristics and their primary renal disease are shown on Table 1.

Patients with a history of nephrotic syndrome, diabetes mellitus, alcohol consummation, systemic illness, or any other disease that might influence lipid metabolism were excluded. None of the patients received lipid lowering drugs, L-carnitine or β-blockers in the 3 months prior to entering the study. However, 25 patients with hypertension received treatment angiotensin-converting enzyme inhibitors. Patients received no other medications except multivitamins, calcitriol phosphate binders, and/or iron. Patients maintained their habitual diets (35 kcal/kgbw, protein intake 1–1.2 g/kgbw, fats <35% caloric intake) with sodium and potassium restriction during the previous 3 months. They had low habitual consumption of foods

Table 1
Patients' characteristics

No. (M/F)	37 (21/16)
Age (years)	52 ± 10
Duration (years)	6 ± 3
BMI (kg/m^2)	23.65 ± 3.52
Hypertension (yes/no)	25/12
Primary causes of end-stage renal disease (%)	
Glomerulonephritis	11 (30)
Pyelonephritis	11 (30)
Polycystic kidney disease	7 (19)
Nephropathies endemic	6 (16)
Nephroangiosclerosis	2 (5)

containing soy, fish intake once a week, and no dietary supplementation of oil rich in long-chain fatty acid (fish, sesame, or linseed oil) as determined by diet assessment made at the time of recruitment. Blood samples were drowning from their fistula before dialysis and after a 12- to 14-h overnight fast. Blood samples were collected from 29 healthy volunteers (control group) from medical staff and blood donors (17 males, 12 females, mean age 55 ± 9 years, range 44–62) after a 12-h overnight fast. All patients signed an informed consent document. Study protocol was approved by the Medical Ethics Committee (Institute for Medical Research, Belgrade) and conducted in line with the principles of good scientific practice.

Biochemical Determination

Serum samples were prepared by 4°C centrifugation of venous blood collected after 12- to 14-h fast. Total serum cholesterol and triglyceride level were measured spectrophotometrically by using a colorimetric enzymatic reaction (EliTech Diagnostic, Sées, France). Serum albumin concentration was determined by using bromcresolgreen reagent (EliTech Diagnostic). HDL-cholesterol was determined by measuring the cholesterol in supernatant liquid after serum precipitation with phosphotungstic acid, magnesium chloride, and LDL-cholesterol was estimated by using Friedewald formula.[18,19] Total phospholipids were determined by the method of Zilversmit and Davis.[20] C-reactive protein (CPR) level was measured with a ELISA test (EliTech Diagnostic), with rabbit antibodies to human CRP. Values <6 mg/L were considered normal.

Plasma lipids were extracted with chloroform-methanol mixture (2:1 v/v) by Sperry and Brand method.[21] Plasma phospholipids were separated by thin-layer chromatography (TLC) into four fractions [i.e., lyso-phosphatidylcholine (LPC), phosphatidylcholine (PC), sphingophospholipids (SPL), and phosphatidylethanolamine

(PE)] in a one-dimensional TLC system using chloroform:methanol:20% ammonia solution (70:25:5, v/v/v) as the eluting solvent.

After removing the plasma and buff coat, packed erythrocytes (2–3 g) were washed three times with 5 mL of 0.9% NaCl and separated by centrifugation (3000 r.p.m. for 10 min) before homogenization. The washing solution was discarded, and the global fraction was frozen at −20°C for 1 h to produce rupture of the erythrocyte membrane. Red blood cell membrane lipids were extracted by Harth's method.[22] The phospholipid fraction was isolated from the extracted lipids by one-dimensional TLC in a neutral solvent system hexane-diethyl ether-acetic acid (87:12:1 v/v) using Silica Gel GF plates (C. Merck, Darmstadt, Germany).

Methyl esters of phospholipid fatty acids (from plasma and erythrocytes) were prepared by methods reported previously.[23] Fatty acid methyl esters derivatives formed from isolated plasma phospholipid fraction were separated by gas chromatography using a Varian GC (model 3400, Varian Associates) as described.[24] Individual fatty acid methyl esters in the sample were identified from the retention times of authentic standards (Sigma Chemical Co) and/or polyunsaturated fatty acids (PUFA-2) mixture (Supelco Inc., Bellefonte). Peak areas were determined with a Varian 4290 integrator, and the results were expressed as the relative percentage of total identified fatty acids.

Statistical Analyses

All results are expressed as means ± SD. The data were analyzed by using one-way analysis of variance model followed by The Fisher exact test,[25] accepting an alpha level of significance 0.05.

RESULTS

Plasma lipids' profile of 37 HD patients is shown on Table 2. Plasma triglyceride ($p < 0.001$), total cholesterol ($p < 0.05$), and total phospholipids ($p < 0.01$) levels were significantly higher, and HDL-cholesterol levels significantly lower ($p < 0.01$) in HD patients than in the control subjects. Plasma PC and PE concentrations were significantly higher ($p < 0.001$) in HD patients. LDL-cholesterol was not different in plasma of HD patients. Plasma phospholipids distribution (Table 3) showed significantly higher ($p < 0.001$) participation of PC and lower ($p < 0.001$) participation of LPC, SPL, and PE ($p < 0.01$) in HD patients

Plasma and erythrocyte phospholipid fatty acids composition in HD patients is shown in Table 4. These results indicate no difference in plasma phospholipid saturated fatty acids, significantly higher ($p < 0.01$) level of oleic

Table 2
Plasma lipids profile

Plasma lipids (mmol/L)	Hemodialysis patients ($n = 37$)	Control subjects ($n = 29$)
Triglycerides	2.30 ± 0.29***	1.38 ± 0.26
Total cholesterol	5.48 ± 0.89*	5.00 ± 0.64
HDL-cholesterol	1.42 ± 0.44**	1.69 ± 0.33
LDL-cholesterol	3.04 ± 0.81	3.13 ± 0.60
Total phospholipids	3.10 ± 0.66***	2.25 ± 0.35
Phosphatidylcholine	2.16 ± 0.10***	1.45 ± 0.06
Lysophosphatidylcholine	0.21 ± 0.03	0.21 ± 0.02
Sphingophospholipids	0.57 ± 0.04	0.46 ± 0.02
Phosphatidylethanolamine	0.15 ± 0.02***	0.12 ± 0.01

The values are means ± SD.
*Significantly different compared to C group, $p < 0.05$.
**Significantly different compared to C group, $p < 0.01$.
***Significantly different compared to C group, $p < 0.001$.

Table 3
Plasma phospholipids distribution

Phospholipids (%)	Hemodialysis patients ($n = 37$)	Control subjects ($n = 29$)
Phosphatidylcholine	69.60 ± 3.14***	64.40 ± 2.82
Lysophosphatidylcholine	6.96 ± 0.86***	9.41 ± 0.82
Sphingophospholipids	18.45 ± 1.38***	20.70 ± 0.99
Phosphatidylethanolamine	4.99 ± 0.82**	5.49 ± 0.38

The values are means ± SD.
*Significantly different compared to C group, $p < 0.05$.
**Significantly different compared to C group, $p < 0.01$.
***Significantly different compared to C group, $p < 0.001$.

(18:1 n-9), and significantly lower ($p < 0.05$) levels of eicopentaenoic (20:5 n-3 EPA) and docosahexaenoic (22:6 n-3 DHA) acids in HD patients. MUFA (monounsaturated fatty acids) was significantly higher ($p < 0.05$), and PUFA n-3 significantly lower ($p < 0.05$) than in the control subjects. In HD patients, PUFA n-6 was significantly lower ($p < 0.05$). However, the fatty acid composition of erythrocyte phospholipid in HD patients was also altered (Table 4). EPA and DHA levels were significantly lower ($p < 0.05$), and consistent with this, PUFA n-3 levels were significantly lower ($p < 0.05$) than in control subjects.

DISCUSSION

Dyslipidemia is one of the main risk factors of cardiovascular complications in patients with chronic renal disease.[3,4] There is increasing evidence that hyperlipidemia is a contributing factor to the progression of chronic renal

Table 4
Plasma and erythrocyte phospholipid fatty acids composition (mol%) in hemodialysis patients

Fatty Acids	Plasma phospholipids		Erythrocyte phospholipids	
	HD patients ($n = 37$)	Controls ($n = 29$)	HD patients ($n = 37$)	Controls ($n = 29$)
16:0	28.09 ± 3.34	26.46 ± 2.44	21.63 ± 1.85	22.42 ± 2.59
18:0	15.67 ± 3.59	16.14 ± 2.41	19.28 ± 1.49	18.71 ± 1.71
16:1 n-7	0.41 ± 0.26	0.29 ± 0.16	—	—
18:1 n-9	13.05 ± 2.21**	11.71 ± 1.24	17.86 ± 1.77	17.53 ± 2.70
18:2 n-6	25.50 ± 2.88	26.47 ± 2.78	14.80 ± 2.01	15.53 ± 1.83
20:3 n-6	1.98 ± 0.50	2.42 ± 0.71	1.26 ± 0.41	1.24 ± 0.44
20:4 n-6	11.06 ± 2.21	11.56 ± 2.32	15.29 ± 1.83	16.25 ± 2.03
22:4 n-6	0.44 ± 0.11	0.39 ± 0.19	3.54 ± 0.86	3.53 ± 1.23
20:5 n-3	0.26 ± 0.14*	0.36 ± 0.14	0.23 ± 0.06*	0.49 ± 0.14
22:5 n-3	0.54 ± 0.14	0.57 ± 0.10	1.21 ± 0.40	1.29 ± 0.46
22:6 n-3	3.00 ± 0.90*	3.63 ± 1.12	4.33 ± 0.83*	5.32 ± 1.10
SFA	43.77 ± 5.23	42.60 ± 3.10	40.91 ± 2.97	41.13 ± 4.84
MUFA	13.45 ± 2.50*	12.00 ± 1.74	17.86 ± 1.77	17.53 ± 2.70
PUFA n-6	38.98 ± 3.28*	40.84 ± 2.86	34.87 ± 3.50	36.59 ± 2.39
PUFA n-3	3.80 ± 1.19*	4.56 ± 1.35	5.96 ± 0.88*	6.93 ± 1.27
n-6/n-3	9.59 ± 1.32	8.82 ± 1.58	5.93 ± 0.91	5.46 ± 1.06

The values are means ± SD.
*Significantly different compared to C group, $p < 0.05$.
**Significantly different compared to C group, $p < 0.01$.

diseases.[1,26] In our study, patients on hemodialysis have higher levels of plasma triglycerides, total cholesterol, and lower levels of HDL-cholesterol. Even though low HDL-cholesterol levels are always associated with hypertriglyceridemia,[27] it is reasonable to argue that low HDL-cholesterol is strictly linked to the changes in triglyceride metabolism.

Abnormal phospholipid metabolism may play an important role in the progression of atherosclerosis in patients with end-stage renal disease.[28,29] Plasma phospholipids distribution was altered in HD patients in our study. In the present data, we have found a significant decrease in plasma SPL distribution in HD patients. The in vitro study by Zager[30] showed that SPL could directly decrease cell/membrane damage. In fact, Zager argued that SPL could extend potent antiproliferative effects in the setting of subendotel tubular damage in renal failure. SPL serves as a critical modulator of membrane fluidity.[31] The importance of phospholipids for the structure and integrity of cellular membranes suggests that many functional disturbances in renal failure may be related to changes in phospholipids distribution and fatty acid composition. In our study, disturbance in fatty acid metabolism in HD is evident. The level of oleic acid (one of MUFA) in plasma phospholipids was elevated, and the levels of PUFA, especially EPA and DHA, two n-3 PUFA, was reduced in plasma and erythrocyte phospholipids. Our results showing the increase of oleic acid and total MUFA

in patients with chronic renal failure agree with previous reports.[32,33] De Gomez Dumm et al.[32] found decreased plasma PUFA in patients on hemodialysis; the lipid composition abnormality persisted after 18 months and became more notorious after 30 months. Varga et al.[33] concluded that the relative abundance of saturated fatty acids (SFA) and MUFA in plasma of HD patients is associated with concomitant lipid disorders and cardiomyopathy, whereas the low relative abundance of PUFA was common in all HD patients. Conversely, Reaven et al.[34] reported that the high oleic acid content in patients with chronic renal failure might exhibit protective roles.

Studies examining the relationship between blood individual fatty acid with serum triglycerides and total cholesterol are not consistent.[35,36] Grimsgarard et al.[37] reported that the concentration of EPA and DHA and total n-3 showed inverse association with serum triglycerides, whereas oleic acid was positively associated with triglyceride concentrations. Plasma triglyceride concentration represents a functional indicator of n-3 PUFA because n-3 PUFA exerts a consistent hypertriglyceridemic effect, which is dose dependent and persistent.[38] Decrease in PUFA, especially n-3 may be a risk factor for depression often present in patients on hemodialysis.[39] Disturbances in fatty acid metabolism in HD patients in our study (increased oleic acid, decreased EPA and DHA) are associated with high triglyceride levels. Our results showed abnormal lipid metabolism (triglycerides and phospholipids)

and deficiency of n-3 PUFA that may indicate the risk and/or progression of atherosclerosis. In addition, published data[40] suggested that a high proportion of n-3 PUFA in red blood cell membranes is associated with a reduced risk of primary cardiac arrest.

In our study, PUFA n-3 (EPA and DHA) decreased in erythrocyte phospholipids (PUFA n-6 also decreased but not significantly) and may be connected with lower membrane fluidity in patients with chronic renal failure.[41] This is because the length and degree of unsaturation of the membrane phospholipid fatty acid are the main determinants of fluidity.[42] Peuchant et al.[43] found that erythrocytes of patients with chronically renal failure showed increased lipid peroxidation associated with a reduction in long-chain PUFA. Abnormal fatty acid metabolism may contribute to clinical problems such as itching, pruritus, abnormal perspiration, susceptibility to infection, delayed wound healing, anemia, and increased hemolysis,[15,38] as seen in patients on hemodialysis.

Being overweight and hyperlipidemia are established risk factors in the general population, whereas lower body mass index and lower plasma cholesterol have been shown to be risk factors for cardiovascular mortality in end-stage renal disease.[39,44] Long-term therapy and inadequate diet regimen in end-stage renal disease (terminal phase) lead to weight loss. Dialysis procedure may accentuate malnutrition by removing important nutrients. In addition, malnutrition is characterized by not only impaired lipid metabolism but also results in modification of plasma and erythrocyte fatty acid profile. It is interesting that the studied patients on HD who have adequate nutrition status also show a deficit of essential PUFA, particularly n-3 PUFA. Because composition of fatty acids of serum and erythrocyte phospholipids clearly reflects dietary habits,[45] we suggest that more attention be given to the content and composition of dietary fat to prevent distribution in fatty acid metabolism in all patients with end-stage renal disease and not only in malnutrition ones.

In summary, plasma phospholipids fatty acid profile in our patients on hemodialysis indicate the necessity of nutrition care programs and the use of appropriate diet with adequate n-6/n-3 fatty acid ratio. Well-designed and controlled studies are needed to determine if supplementation might lead to prevention or regression of atherosclerosis and could remove some clinical problems in hemodialyzed patients.

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