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CLINICAL STUDY

Improvement in cardiovascular risk markers by the combined effect of natural polyphenols and vitamins in patients after kidney transplantation

SZENTESIOVA Zuzana[1]*, TREBATICKY Branislav[2]*, ZILINSKA Zuzana[2,4], BREZA Jan[2], ORAVEC Stanislav[3], ORSZAGHOVA Zuzana[1], MUCHOVA Jana[1]

Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovakia. **jana.muchova@fmed.uniba.sk**

ABSTRACT

OBJECTIVES: The aim of this study was to evaluate the potential of supportive therapy by natural polyphenols combined with vitamins C and E on kidney function and risk factors of cardiovascular diseases in renal transplant recipients (RTR).

BACKGROUND: Transplant patients have an altered lipid profile associated with the development of cardiovascular disease, which is a major cause of graft loss and mortality in patients.

METHODS: The study included 29 renal transplant recipients with mean graft function levels. The lipoprotein (atherogenic and non-atherogenic) subfractions were identified and quantified in plasma by polyacrylamide gel electrophoresis.

RESULTS: After supplementation, glomerular filtration rate (GFR) was increased by 8 %, serum creatinine was decreased by 6.7 % and significant changes were found in atherogenic LDL subfractions. The effect of supplementation was observed in arylesterase and lactonase activities of paraoxonase 1 which increased by 9.3 % and 8.1 %, respectively. In addition, significantly decreased levels of neopterin (by 16 %) and asymmetric dimethylarginine (ADMA) (by 7.9 %) were found.

CONCLUSION: We could summarize that supportive therapy improves the renal function (GFR, serum creatinine), and reduces the risk of cardiovascular disease by affecting important risk markers of atherosclerosis (lipid profile, paraoxonase 1 activity, neopterin and ADMA) in RTR *(Tab. 4, Fig. 1, Ref. 53)*. Text in PDF *www.elis.sk*

KEY WORDS: kidney transplantation, lipoprotein subfractions, Lipoprint System, Pycnogenol.

Abbreviations: CVD – cardiovascular disease, PYC – Pycnogenol, TCH – total cholesterol, VLDL – very low-density lipoproteins, LDL – low-density lipoproteins, HDL – high-density lipoproteins, IDL1 to 3 – intermediate-density lipoproteins, LDL 1 to 7 – subfractions of LDL, L-HDL – large HDL subfraction (L-HDL 1 to 3); I-HDL – intermediate HDL subfraction (I-HDL4 to 7); S-HDL – small HDL subfraction (S-HDL8 to 10), ADMA – asymmetric dimethylarginine, NP – neopterin, PON 1 – paraoxonase 1, CyA – cyclosporin A, TAC – tacrolimus, SIR – sirolimus, MMF – mycophenolate mofetil, RTR – renal transplant recipient, GFR – glomerular filtration rate, KT – kidney transplantation, AI_1, AI_2 – atherogenic indexes

Introduction

The preferred therapy for patients with end-stage renal disease is kidney transplantation (KT). Although transplant patients have a higher risk of being susceptible to infections and an increased risk of developing malignant diseases, the main causes of mortality are cardiovascular diseases. The atherogenic risk to transplant patients is due to the summation of factors such as dialysis, immunosuppressive drugs, but also the high frequency and accumulation of atherogenic risk factors before and after transplantation (1, 2). Optimal control of cardiovascular risk factors, especially hyperlipidemia, is important in the long-term management of these patients, as lipid abnormalities, namely elevated levels of total cholesterol, VLDL and LDL-cholesterol, as well as elevated triglycerides and apolipoprotein B are common findings in kidney transplant patients (3).

[1]Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Bratislava, Slovakia, [2]Department of Urology with Center for Kidney Transplantations, Faculty of Medicine, University Hospital, Bratislava, Slovakia, [3]1st Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovakia, and [4]5th Department of Internal Medicine, Faculty of Medicine, University Hospital Bratislava, Bratislava, Slovakia

Address for correspondence: Jana MUCHOVA, Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University, Sasinkova 2, SK-811 08 Bratislava, Slovakia.

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* equal contribution

Risk factors for chronic kidney disease are also individual atherogenic subfractions of plasma lipoproteins, small LDL (LDL-3 to 7) and small HDL (S-HDL8 to 10) due to their low receptor recognition, increased oxidation and acetylation capacity, easier penetration into the subendothelial space, and formation of cholesterol deposits (4). Dyslipidemia remains one of the most common and modifiable risks (5, 6).

An important contributor to dyslipidemia in transplant recipients is the metabolic side effect of immunosuppressive drugs, which can alter lipoproteins and their metabolism (5, 6). Among the posttransplant immunosuppressants used, cyclosporin A (CyA) is responsible for increasing LDL-CH and triglyceride levels and lowering HDL-CH serum levels (3). The atherogenic potential of immunosuppressants decreases in the order of steroids > CyA > sirolimus > tacrolimus > mycophenolate mofetil (MMF). MFF has minimal effects on lipid levels alone, but its effects in combination with corticosteroids and CyA may be different (7).

One of the main conditions associated with increased cardiovascular morbidity and mortality is chronic inflammation. Inflammation in conjunction with endothelial dysfunction and oxidative stress in transplant patients adversely affects cardiovascular outcomes (8). In addition to cardiovascular disease, inflammation is one of the major factors in the progression of other chronic diseases such as diabetes, obesity, arthritis, autoimmune diseases, and cancer (9). Inflammation and related adverse effects have led to the development of innovative additional therapeutic targets derived from natural sources. The treatment with natural antioxidants could contribute to the suppression of chronic diseases associated with inflammation, oxidative stress, and development of atherosclerosis (10). In our previous *in vitro* study (11) we found that polyphenolic extract Pycnogenol (PYC) (12) used together with ascorbic acid or Trolox at appropriate concentrations may provide efficient antioxidant protection to the organism. PYC with Trolox exerted an additive effect, while PYC with ascorbic acid had a synergistic effect. These effects could make it possible to administer vitamin C at lower doses because in transplant patients, even at a dose of 500 mg/day, vitamin C can lead to secondary oxalosis (13).

The aim of this study was to evaluate the potential effect of 3-month supportive therapy with natural polyphenolic extract PYC in combination with vitamins C and E on (i) clinical-biochemical parameters of transplanted kidney function (serum creatinine and urea concentration, glomerular filtration rate), (ii) lipid profile, including subfractions of lipoproteins determined by Lipoprint LDL and HDL System, (iii) cardiovascular risk markers (c-reactive protein (CRP), neopterin (NP), asymmetric dimethylarginine (ADMA), homocysteine (HCy), paraoxonase 1 activity (PON 1)) and (iv) immunosuppressants level in renal transplant recipients (RTR), and their mutual associations.

Material and methods

Study population and intervention

A cohort of 29 patients after kidney transplantation (14 females and 15 males; average age 55.21 ± 12.42 years) was identified. Patients were 4 to 15 years after kidney transplantation. Kidney transplant recipients showed a mean graft function, expressed as GFR, 1.13 mL/s per 1.73 m^2 (0.73–1.75 mL/s per 1.73 m^2) and mean serum creatinine levels of 96.52 μmol/L (60.5–144 μmol/L) while the other biochemical parameters were in the reference range. Patients did not take other vitamin supplements. All patients were treated with immunosuppressant tacrolimus (TAC) (25 patients), cyclosporine A (2 patients) and sirolimus (SIR) (2 patients) in combination with corticosteroids – prednisone (21 patients), and MMF (28 patients). Tacrolimus was administered in doses as follows: < 3 ng/mL (18 patients), 3–5 ng/mL (3 patients), 5–8 ng/mL (2 patients), > 8 ng/mL (2 patients) while average level during the study was 6.00–6.28 ng/mL. All patients also received corticosteroids (2.5–10 mg/day) and MMF (360–2000 mg/day). The dose of immunosuppressants was adjusted during the study according to their actual level: the dose of TAC in 13 patients, corticosteroids in 2 patients and MMF in 3 patients. All patients signed the informed consent for participation in the study.

The causes of end-stage renal disease and consequent KT in the recruited patients were as follows: chronic glomerulonephritis, chronic tubulointerstitial nephritis, polycystic kidney disease, Henoch – Schönlein purpura, chronic mesangioproliferative glomerulonephritis, IgA nephropathy, hypertensive nephrosclerosis, chronic pyelonephritis, focal segmental glomerulosclerosis, and obstructive nephropathy.

The renal transplant recipients have been daily administered a mixture of natural polyphenolic extract Pycnogenol (50 mg), vitamin C (250 mg) and vitamin E (200 mg) for 3 months. Compliance with the intervention was assessed to be 95 %.

During the study period, participants were examined, and biological samples were taken at baseline (0), after three months of supplementation (1) and after 1 month of wash-out period (2).

Determination of biochemical parameters

The concentrations of biochemical parameters were analyzed by standard methods in an accredited Clinical Biochemistry and Haematology Laboratory SYNLAB Slovakia. Auto*Mate* 2500 Family Sample Processing Systems (Beckman Coulter Inc.) was used.

Determination of lipoprotein subfractions

The determination of lipoprotein subfractions is based on electrophoretic separation of lipoproteins on a polyacrylamide gel (Lipoprint System, Quantimetrix Corp., CA, USA).

Determination of inflammatory markers

The concentrations of inflammatory markers were determined using the commercial ELISA kits. Neopterin was determined using the ELISA kit (No. RE59321) from IBL International GmbH, Germany and asymmetric dimethylarginine was determined using the ELISA kit (No. EA201/96) from DLD Diagnostika GmbH, Germany.

Tab. 1. Effect of supplementation on serum cholesterol, lipoproteins and LDL subfractions.

Parameter	Before suppl. (baseline) 0	After 3 months of suppl. 1	p (0/1)	After 1 month of wash-out period 2	p (0/2)
TCH (mmol/L)	4.83±0.45	4.54±0.55	**0.0244**	4.57±0.69	*0.0832*
VLDL (mg/dL)	43.1±10.64	39.17±11.36	*0.0594*	41.00±11.43	0.3007
IDL1 (mg/dL)	28.93±5.84	23.10±6.56	**< 0.0001**	24.55±5.82	**0.0009**
LDL-1 (mg/dL)	35.76±7.51	32.66±8.33	**0.0373**	32.83±9.11	*0.0580*
LDL-CH (mg/dL)	103.31±11.84	97.54±14.52	*0.0625*	95.46±16.79	**0.0208**
AI_1	4.96±1.17	4.76±1.16	*0.0696*	4.68±1.12	**0.0097**

Values are expressed as mean ± SD. TCH – total cholesterol, VLDL – very low-density lipoproteins, IDL1, IDL2 –intermediate-density lipoproteins, LDL-1/-2 – subfractions of total LDL cholesterol, HDL – high-density lipoproteins, LDL – low-density lipoproteins, p – significance

Tab. 2. HDL subfractions in renal transplant recipients.

Large HDL subfractions		Intermediate HDL subfractions		Small HDL subfractions	
L-HDL1	12 (14.5–9.5)	I-HDL4	12 (12–9)	S-HDL8	6 (8–5)
L-HDL2	12 (14–8)	I-HDL5	9 (11–8)	S-HDL9	5 (7–4)
L-HDL3	9 (12–7)	I-HDL6	21 (24–18)	S-HDL10	6 (9–3.5)
		I-HDL7	7 (9–6)		
L-HDL total	35 (39–24)	I-HDL total	49 (53–44)	S-HDL total	17 (24–13)

Values are expressed as median (upper-lower quartile) in the area (%) under the curve. L-HDL – large HDL subfraction; I-HDL – intermediate HDL subfraction; S-HDL– small HDL subfraction

Tab. 3. Correlations of atherogenic index AI_1 with lipoproteins and LDL/HDL subfractions at Baseline.

Lipoproteins and LDL subfractions				HDL subfractions			
AI_1 vs	n	r	p	AI_1 vs	n	r	p
VLDL	26	**0.684**	**< 0.001**	L-HDL1	26	**-0.698**	**< 0.001**
IDL2	26	**0.464**	**0.017**	L-HDL2	26	**-0.635**	**< 0.001**
IDL3	26	**-0.462**	**0.018**	L-HDL3	26	**-0.723**	**< 0.001**
LDL-1	25	**-0.462**	**0.020**	L-HDL total	26	**-0.725**	**< 0.001**
LDL-2	25	**0.811**	**< 0.001**	I-HDL4	26	**-0.611**	**0.001**
HDL-CH	26	**-0.909**	**< 0.001**	I-HDL6	26	**0.668**	**< 0.001**
LDL-CH	26	**0.404**	**0.041**	I-HDL7	26	**0.697**	**< 0.001**
				I-HDL total	26	**0.525**	**< 0.001**
				S-HDL8	26	**0.675**	**< 0.001**
				S-HDL9	26	**0.689**	**< 0.001**
				S-HDL10	25	**0.666**	**< 0.001**
				S-HDL total	26	**0.695**	**< 0.001**

AI_1 – atherogenic index (AI_1 = total cholesterol (mmol/L)/HDL cholesterol (mmol/L)), VLDL – very low-density lipoproteins, IDL2, IDL3 – intermediate-density lipoproteins, LDL-1, LDL-2 – subfractions of LDL cholesterol, HDL – high-density lipoproteins, LDL – low-density lipoproteins, L-HDL –large HDL subfraction; I-HDL – intermediate HDL subfraction; S-HDL– small HDL subfraction, n – number of subjects, r – correlation coefficient, p – significance

Determination of paraoxonase 1 activities

The arylesterase (PON A) and lactonase activity (PON L) of paraoxonase 1 were determined according to Muchová et al (14).

Statistical analysis

For statistical analysis, we employed the statistical program StatsDirect version 2.7.9. Data are presented as mean±SD or median (upper-lower quartile). p values lower than 0.05 were considered statistically significant. The data normality was tested by Shapiro-Wilk test. In case of normal data distribution, the independent sample t-test was used, and for non-normally distributed data, the Mann-Whitney U test was used. Correlations between the selected biochemical parameters, inflammatory markers, and individual LDL subfractions were analyzed by Pearson's test, and for HDL subfractions, Spearman's test was used. Correlation analyses were performed by the statistical program IBM SPSS ver. 21. For a graphical representation of data Excel 2016 (Microsoft Co.) was used.

Results

After 3 months, a significant increase in GFR as compared to baseline (1.22±0.28 vs 1.13±0.25 mL/s/1.73 m^2, p = 0.0017) and a decrease in S-CREA concentration (90.10±17.66 vs 96.52±17.95 μmol/L, p = 0.0004) were observed. TCH, a complex spectrum of lipoproteins, and LDL lipoprotein subfractions determined by the Lipoprint LDL System are shown in Table 1. Before the supplementation, TCH was increased over the reference range (2.9–5.0 mmol/L) in 7 patients while the average value of all patients fell into the physiological range. The Lipoprint analysis showed that in all patients (except one), the VLDL level had increased above the reference level (≤ 22 mg/dL) during the supplementation period. Similarly, 27 patients at baseline showed IDL1 values (atherogenic subfraction) to be higher than the reference levels for this parameter (≤ 23 mg/dL). As we were interested in the atherogenic phenotype of patients in terms of atherogenic indexes, we calculated them as AI_1 = TCH / HDL-CH and AI_2 = (TCH – HDL-CH)/HDL-CH. In the case of AI_1, 14 of 29 patients showed elevated values (> 4.85), i.e., a higher risk of CVD at baseline. After supplementation, CVD risk was reduced in 10 of them.

The results of the HDL subfractions analysis at baseline are shown in Table 2. We observed total large HDL-CH in 9 patients and total intermediate HDL-CH in 22 patients to be below the reference levels.

Subsequently, we performed a correlation analysis between monitored parameters in relation to atherogenic index AI_1 (Tab. 3).

CRP levels and NP, ADMA and HCy concentrations were studied as additional risk factors for CVD. CRP level at baseline was in the reference range (< 5.0 mg/L) while the concentrations of NP, ADMA and HCy were increased over the reference ranges (> 8.7 nmol/L for NP; > 0.76 μmol/L for ADMA and > 13.0 μmol/L for HCy). After 3 months of supplementation, we found significantly decreased levels of NP (13.79±4.99 nmol/L vs 11.58±6.14 nmol/L, p=0.0029), as well as ADMA (0.89±0.25 μmol/L vs 0.82±0.21 μmol/L, p=0.028).

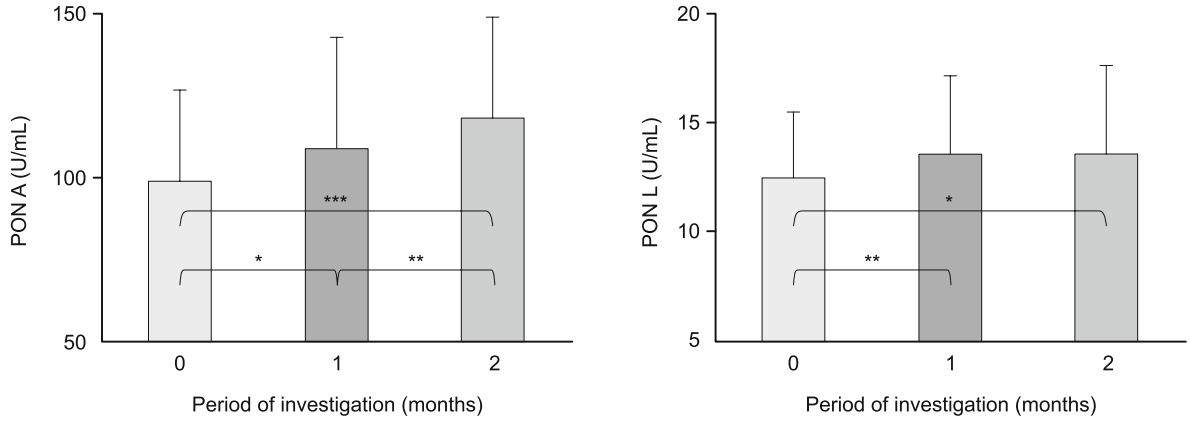

Fig. 1. The arylesterase (PON A) and lactonase (PON L) activities of paraoxonase-1. Values are presented as mean ± SD. 0 – before supplementation, 1 – after three months of supplementation and 2 – one month after termination of supplementation (wash-out period). * (p < 0.05), ** (p < 0.01), * (p < 0.001).**

The arylesterase and lactonase activities of the antioxidant enzyme paraoxonase (PON1) are shown in Figure 1. After 3 months of supplementation, we found statistically higher PON A and PON L activities.

Within the HDL subfractions at baseline, significant correlations were found mainly with paraoxonase 1 (PON 1), which are shown in Table 4. PON 1 as an enzyme located in HDL showed a significant positive correlation of its arylesterase as well as lactonase activities with total HDL-CH. However, HDL subfractions alone did not correlate with PON 1. We observed correlations of PON L with individual HDL subfractions only 1 month after the end of supplementation (Tab. 4).

We also studied the correlations between used immunosuppressant concentrations (TAC, prednisone and mycophenolate mofetil) and lipid subfractions, cardiovascular and biochemical markers via correlation analysis. We confirmed the atherogenic effect of TAC and prednisone, which reported a negative correlation with antiatherogenic L-HDL1 and L-HDL2 subfractions (r=–0.408 to –0.476; p < 0.05) and a positive correlation with atherogenic S-HDL8 to 10 subfractions (r=0.412–0.444; p < 0.05), while out of the LDL particles, only the level of VLDL was positively correlated (r=0.627, p=0.001). On the other hand, MMF demonstrates an antiatherogenic effect, which we confirmed by a positive correlation with L-HDL1 and negative correlation with I-HDL7, S-HDL8 and 10, which subsequently conditioned a positive correlation with L-HDL total and negative correlation with S-HDL total. As to the cardiovascular markers, a correlation was found only between TAC and AI_1 (r=0.532, p=0.011), and as to the biochemical parameters, associations were found between TAC and S-CREA (r=0.5, p=0.011), as well as between TAC and U-CREA (r=0.425, p=0.038). Prednisone also positively correlated with the inflammatory marker ADMA (r=0.582; p=0.018).

Tab. 4. Correlations between HDL subfractions and cardiovascular/inflammatory markers.

Baseline (0)				After 1 month of wash-out period (2)			
	n	r	p		n	r	p
PON A vs HDL-CH	26	**0.444**	**0.023**	PON L vs HDL-CH	29	**0.535**	**0.003**
PON L vs HDL-CH	29	**0.520**	**0.004**	PON L vs L-HDL2	29	**0.492**	**0.007**
PON A vs HCy	16	**–0.547**	**0.028**	PON L vs L-HDL3	29	**0.630**	**< 0.001**
PON L vs HCy	19	–0.420	*0.073*	PON L vs L-HDL total	29	**0.419**	**0.024**
CRP vs S-HDL10	27	**0.465**	**0.015**	PON L vs I-HDL4	29	**0.512**	**0.005**
CRP vs S-HDL total	28	**0.382**	**0.045**	PON L vs I-HDL6	29	**–0.441**	**0.017**
PON A vs S-CREA	26	**–0.389**	**0.050**	PON L vs I-HDL7	29	**–0.422**	**0.022**
NP vs CRP	26	0.349	*0.081*	PON L vs S-HDL10	28	**–0.420**	**0.026**
				PON L vs S-HDL total	29	**–0.414**	**0.025**

PON A – arylesterase activity of paraoxonase-1, PON L – lactonase activity of paraoxonase-1, HCy – homocysteine, CRP – high sensitivity c-reactive protein, L-HDL – large HDL subfraction; I-HDL – intermediate HDL subfraction; S-HDL– small HDL subfraction, n – number of subjects, r – correlation coefficient, p – significance

Discussion

We monitored the effect of a 3-month supplementation with natural polyphenol PYC extract in combination with vitamins C and E on renal function and atherosclerosis risk factors in renal transplant patients. We found a significant improvement in renal function, lipid profile, which is also associated with cardiovascular risk assessed using an atherogenic index and reduction in selected inflammatory parameters. Since administered supplements do not affect immunosuppressive levels, adequate immunosuppressive therapy can be provided to renal transplant recipients (RTR).

After successful kidney transplantation, the renal functions of the body are restored. The patients in our study had on average a good graft function after kidney transplantation while three-month supplementation with PYC in combination with vitamins C

and E even improved these renal function parameters. The rate of glomerular filtration increased, which is consistent with the randomized placebo-controlled study of Blackhall et al (15) who observed an improvement in glomerular filtration in recipients of transplanted kidneys after administration of a combination of antioxidants (800 IU (536 mg) of vitamin E, 500 mg of vitamin C and 6 mg of beta-carotene). An increase in GFR also leads to a decrease in serum creatinine, as observed by Loong et al (16) who found a decrease in serum creatinine by more than 20 % in a group of 5 patients supplemented with vitamin C and/or E (500 mg vit C and/or vit E), while in our study of 29 patients, the effect on creatinine concentration was not as significant, which was probably due to different doses of administered supplements. However, when comparing our work with other studies (15, 16) we used a lower dose of vitamin C (250 mg/d), which is safer way for transplant patients to achieve improved clinical parameters.

It is common in renal transplant patients to find lipid abnormalities associated with the development of post-transplantation CVD. Recent studies show that the incidence of cardiovascular events in transplant patients is about 6 times higher than in the general population. Dyslipidemia remains one of the most significant risks of atherosclerosis (4, 5).

In our study, in addition to the determination of total cholesterol and lipoproteins, a complex spectrum of LDL/HDL lipoprotein subfractions were investigated. Although the total cholesterol and LDL-CH levels were in the reference range, we found elevated atherogenic VLDL and IDL1 levels as well as reduced total HDL-CH (below 40 mg/dL), indicating an atherogenic profile. Similar results were obtained in the study of Breza et al (17). The calculated values of AI_1 and AI_2 confirm these findings even though atherogenic subfractions of LDL-3-7 were not found in our sample of patients at all.

Elevated VLDL levels and decreased HDL-CH levels despite normal TCH levels were described in the study of Riella et al (18). In our study, we found a normal TCH level but increased concentrations of atherogenic VLDL and IDL1 at baseline. After 3 months of supplementation, a decrease in the levels of TCH, VLDL, IDL1 and LDL-1 was observed. The biomodulatory effect of PYC on lipoprotein levels has been demonstrated in several studies. Devaraj et al (19) found a significant reduction in LDL-CH and an increase in HDL-CH. We also confirmed the beneficial effect of PYC in our previous study of Trebaticky et al (20), which was focused on erectile dysfunction. PYC supplementation reduced TCH and LDL-CH levels, while HDL-CH and triacylglycerol levels remained unchanged. A beneficial effect on total cholesterol and HDL-CH levels in patients with hypercholesterolemia was also found in the case of vitamin C administration. Although the epidemiological studies suggest a possible reducing effect of higher intakes of vitamins C, E, and β-carotene on cardiovascular risk in the population, clinical studies on the protective effect of alone ascorbate against CVD have not led to a clear conclusion. A decrease in the levels of TCH or LDL-CH, even in high-risk patients, is associated with a statistically and clinically significant decrease in cardiovascular mortality (21).

High-density lipoprotein (HDL) is considered to be one of the protective factors against atherosclerosis. Its function lies in its active participation in the re-transport of cholesterol. However, most recent evidence reveals a controversy in the anti-atherogenic effect of HDL and pointed out the importance of HDL subfraction analysis in dyslipidemia diagnosis and treatment (22).

Our work is the first study that provides a panel of HDL subfractions levels in the patients after kidney transplantation. We did not observe the effect of supplements administration on HDL-CH levels while the levels of individual HDL subfractions were not affected either. Nevertheless, the correlation analysis confirmed changes in their function, which may be related to the remodeling of HDL particles as described by Contreras-Duarte et al (23) in a study with supplementation with vitamins C and E. In addition to lipid remodeling, vitamins C and E support further modifications of HDL-CH by increasing the amount of PON 1 and Apo D which correlate with improved HDL-CH antioxidant activity, thus leading to improved atheroprotective function. Also, Babaev et al (24), in their animal study, suggested that the supplementation with a combination of vitamin C and E improves early atherosclerosis. In addition to the effect of vitamins on PON 1 activity, several studies have also found a positive effect of polyphenols and anthocyanins on HDL-CH levels and activity of PON 1. This was found to be strongly associated with an enhanced cholesterol efflux capacity of HDL particles (25, 26).

We studied both the arylesterase as well as lactonase activities of PON 1. According to Kennedy et al (27), the reduced arylesterase activity of PON 1 predicts a higher risk of long-term adverse cardiovascular events in patients with chronic kidney disease. PON 1 activity is thought to be increased after kidney transplantation, which we also confirmed in our previous study (28), where we compared these activities in patients on dialysis and in transplant patients. After three months of supplementation with PYC in combination with vitamins C and E, we found an increase in arylesterase and lactonase activities of PON 1.

There is a close physiological relationship between PON 1 and HDL-CH, as PON 1 prevents the oxidation of HDL-CH (29). Correlation analyses revealed several significant positive correlations between PON L and HDL-CH or individual HDL subfractions, or L-HDL and I-HDL4 and I-HDL5, while I-HDL6 and I-HDL7 correlated negatively with PON L, which is consistent with the results of Muchová et al (14) in hypercholesterolemic children, where I-HDL6 and I-HDL7 are not considered atherogenic subfractions. Similarly, the same study (14) showed that none of the HDL subfractions correlated with PON A. In our study, we found correlations of PON L with individual HDL subfractions up to one month after the end of supplementation. The persisting effects of polyphenolic extracts after the wash-out period were confirmed also in other studies (30, 31).

Since the atherogenic indexes more accurately express and define the atherogenic plasma phenotype as compared to the classical biochemical markers, their high correlation with lipoproteins may explain their high predictive value (32). Therefore, in our study, we applied a correlation analysis to determine the relationships between atherogenic indexes AI_1/AI_2 and lipoprotein subfractions.

The improvement in inflammatory markers after kidney transplantation in comparison with the results from patients on dialysis was confirmed already in our previous study (28). Recurrent inflammation and increased oxidative stress are associated with cardiovascular disease and mortality in patients before KT but also after KT.

In our study, we determined increased baseline levels of NP, ADMA and HCy. NP is a sensitive marker of immune system activation (33). Due to the close association between NP and ROS production, the concentration of NP in body fluids is considered an indicator of oxidative stress due to immune activation. As NP is a major risk factor for CVD, there was observed a negative correlation between NP concentration and antioxidants (ascorbic acid, α-tocopherol, lycopene, lutein and zeaxanthin) in patients with and without CVD (34). We achieved a significant post-supplementation reduction in NP levels. The effect of vitamins C and E on the progression of transplant-associated atherosclerosis was studied by Fang et al (35) who concluded that vitamin supplementation slows down the progression of coronary atherosclerosis. No published clinical data on the effects of PYC on NP are available.

We achieved also a significant reduction in ADMA levels, namely by 7.87 %. This was confirmed by several studies which have shown decreased ADMA levels during the first month after transplantation but still elevated as compared to the control population. Due to this decline, NO synthesis increases, and endothelial function improves (36, 37). Additionally, the plasma level of ADMA is a significant risk factor for graft failure, which may predict morbidity, mortality, and worsening of graft function (38). In order to restore endothelial production of NO, many studies have been conducted on the use of antioxidants such as tea and vegetables (39). Vitamin C (40) or vitamin E (41) increase endothelial NO synthesis by stabilizing the NOS cofactor – tetrahydrobiopterin, and by increasing the local bioavailability of NO and thereby slowing the progression of atherosclerosis. Engler et al (42) studied the impact of vitamins C and E on endothelial function. Antioxidant therapy with vitamins C and E restores endothelial function in hyperlipidemic children but without effect on biomarkers of oxidative stress, inflammation or ADMA. A Finnish study by Päivä et al (43) found no effect of the intake of vitamins C and E in food on ADMA levels.

As hyperhomocysteinemia is one of the risk factors of CVD, there is still a debate about the potential proatherogenic effects of HCy alone (44). The results of epidemiological studies do not indicate a significant association between an increase in plasma HCy and change in total plasma cholesterol, but some studies have found a negative correlation with HDL-CH (45, 46, 47). In our study, no correlation was found between HCy and HDL-CH or TCH, respectively. Probably the formation of inflammatory HCy correlates with a decrease in the activity of enzymes associated with HDL-CH, including PON 1 as a multifunctional enzyme, with its antioxidant capacity and ability to detoxify the homocysteine metabolite homocysteine thiolactone (48, 49).

Immunosuppressive therapy also contributes to the development of CVD. The immunosuppressants most commonly used to prevent graft rejection are tacrolimus, mycophenolate mofetil, and prednisone (50). Also, our patients use this triple combination. In some studies, it was found that tacrolimus and prednisone contribute to hyperlipidemia (51, 52) and this was confirmed in our study by correlation analysis. We found negative correlations of tacrolimus and prednisone with non-atherogenic subfractions L-HDL1, L-HDL2 and also total L-HDL and their positive correlation with atherogenic S-HDL8 to10 and total S-HDL. Tacrolimus also correlated positively with S-CREA, which could be explained by the effect of renal metabolism on the pharmacokinetics of tacrolimus (53). On the other hand, the MMF demonstrates an antiatherogenic effect, which we confirmed by a positive correlation with L-HDL1 and a negative correlation with I-HDL7 and S-HDL8 and 10, which subsequently conditioned a positive correlation with total L-HDL and negative correlation with total S-HDL.

Conclusion

In our study, we evaluated the potential of 3-month supportive therapy by natural polyphenols combined with vitamins C and E on the lipid profile of patients after KT, as well as the effect on individual subfractions of LDL- and HDL-lipoproteins and on the atherogenic risk expressed by the indexes AI_1 and AI_2. The combined effect of natural polyphenolic extract and vitamins C and E improved renal functions (increased GFR, decreased serum creatinine) as well as cardiovascular risk markers (decreased atherogenic VLDL, IDL1, neopterin and ADMA, increased paraoxonase 1 activities) in patients after kidney transplantation. The advantage of supplementation by the used antioxidants is supported by the fact that they did not influence the immunosuppressive status of patients. In clinical research, antioxidant therapies require more time to confirm the applicability of various antioxidant agents as effective treatment methods, in particular in the heterogeneous vulnerable populations such as renal transplant patients.

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