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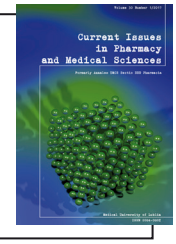

Moderate dyslipoproteinemia induced inflammation and remodeling HDL and VLDL particles in post-renal transplant patients

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ABSTRACT

The aim of this paper was to examine whether moderate dyslipoproteinemia can cause an increase of hsCRP and LPO levels in Tx patients who had received immunosuppressive therapy and were without acute inflammatory diseases. Herein, the lipid levels, hsCRP, LPO, apolipoprotein (apo)B, AI, AII, AIInonB, apoB-containing AII (apoB:AII), apoCIII, apoCIIInonB, apoB:CIII, LCAT level, as well as CETP and PON1 activity were determined. All examined Tx patients had moderate dyslipidemia and slightly increased hsCRP, LPO, apoB:AII and apoCIII levels, but decreased LCAT mass, PON1 activity and lipoprotein ratios. Tx patients with apoAI<150 mg/dl (n=28) had worse lipoprotein profiles than did Tx patients with apoAI>150mg/dl (n=39), but no difference in CETP activity was indicated. Multiple ridge forward regression and Spearman's correlation test were used. The results of the presented study, show for the first time that higher apoAI/apoB and apoAI/apoCIII ratios induced a decrease of the hsCRP concentration. Moreover, the composition of apoCIIInonB, LDL-C and apoAI brought about an increase of LCAT mass and PON1 activity. In Tx patients with lower concentration of apoAI, an increase of concentration of apoB:AII in VLDL generated a mild oxidation of lipoprotein and an elevated concentration of LPO. However, lower ApoAI/apoB ratio resulted in an increase of PON1 activity and apoB, as well as nonHDL-C levels, and in turn, PON1 activity increased LCAT mass. These disorders rearranged the HDL particle, and, simultaneously, remodeled the VLDL particle. This may prevent antioxidant activity, reverse cholesterol transport and accelerate the rejection of the transplant, as well as bringing about cardiovascular diseases in Tx patients with lower apoAI. Such metabolic pathways can be used as potentially novel targets for pharmacological intervention.

INTRODUCTION

Transplanted kidneys are prone to oxidative stress-mediated injury by pre-transplant and post-transplant conditions that cause reperfusion injury or imbalance between oxidants and antioxidants [17]. The inflammatory state plays an important role in causing oxidative stress, especially in end-stage kidney disease (ESRD) and among renal graft recipients [17]. High-density lipoprotein (HDL) protects against atherosclerosis development by a number of mechanisms. Several protein components of HDL can inhibit the oxidation of low density lipoprotein (LDL). These include apolipoprotein AI (apoAI), lecithin: cholesterol acyltransferase (LCAT), and paraoxonase-1 (PON1) [8,9]. However, the proteins interaction in the anti-oxidant activity of HDL is unknown. ApoAII has been shown to directly affect HDL modifications catalyzed by the plasma proteins. ApoAII is less efficient than apoAI at activating LCAT [10,20], and studies have shown that displacement of apoAI in HDL by apoAII inhibits cholesterol esterification. A possible mechanism of the pro-atherogenic action of human apoAII could be the inhibition of reverse cholesterol transport (RCT), depending, at least partly on a marked disease in endogenous

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LCAT [18]. The results concerning the pro- or anti-atherogenic effect of apoAII are still controversial, and further investigation is needed to determine the exact role of apoAII in atherosclerosis [1,3,16]. Atherosclerosis is an inflammatory disease which is believed to be initiated and propagated by the oxidation of LDL in the subintimal space of the vessel wall [3,9]. Inflammation and activation of the immune system may play important roles in atherogenesis. Renal transplant recipients, by the process of receiving an allograft, have an additional activation of their immune system. A small cross-sectional study indicated that renal transplant patients with cardiovascular disease (CVD) had higher high sensitivity C-reactive protein (hsCRP) than patients without CVD [1,3,9,16]. Unfortunately, there is no information about the association of hsCRP and of LCAT, cholesterol ester transfer protein (CETP), lipid hydroperoxide (LPO), PON1 activity, and composition of apoA-containing lipoprotein in HDL and apoB-containing lipoprotein (apoB:AII, apoB:CIII) as triglyceride-rich lipoproteins (TRLs) in VLDL particles in Tx patients without active inflammatory disease.

The aim of this paper was to examine whether moderate dyslipoproteinemia can cause an increase of hsCRP and LPO levels in Tx patients who received immunosuppressive therapy and were without acute inflammatory diseases. Therefore, the lipid levels, hsCRP, LPO, apolipoprotein (apo)B, AI, AII, AIInonB, apoB-containing AII (apoB:AII), apoCIII, apoCIIInonB, apoB:CIII, LCAT level, CETP and PON1 activity were determined.

MATERIAL AND METHOD

Patients

The Tx patients had undergone treatment in the Nephrology Department of the Medical University in Lublin. Ten of the Tx patients had a slight proteinuria, hypertension (n = 43) and cardiovascular disease (n = 1); however, they had no active inflammatory disease, liver disease, malignancy, or diabetes mellitus. The causes of kidney disease in the post-renal transplant patients were: 53 glomerulonephritis, 9 interstitial nephritis, and 5 unknown. The Tx patients with hypertension were using anti-hypertensive medications of either calcium channel blockers or angiotensin converting enzyme antagonists, angiotensin II receptor subtype-1 (AT1) blockers and α-blockers, but no diuretics were used in any of the studied groups. Hyperlipidemic patients were treated with atorvastatin or simvastatin (n = 35), but 32 Tx patients did not receive statin therapy. All Tx patients on immunosuppressive treatment received calcineurine inhibitors and prednisone. Statin treated patients received mainly cyclosporine (60% of Tx patients) as described previously [10]. In this group, the Tx patients received CsA+Myfortic, CsA+Azatiopryne, CsA+CellCept; the patients were given CsA, and Prograf + Myfortic and Prograf+CellCept and CellCept+Myfortic. Tx patients without satin therapy received mainly Prograf (60% of Tx patients) in connection with Myfortic, CellCept and CsA+Myfortic, together with CsA+Azatiopryne and CsA+CellCept [10]. All Tx patients were placed into one of three groups: with apoAI > 150mg/dl and with apoAI < 150mg/dl, and all together. The study was conducted in accordance with the guidelines of the Ethics Committee of the Medical University in Lublin, Poland.

Sample preparation

Whole blood was drawn after 14h of fasting period from the patients. Blood was taken from vein to commercial test tubes. Red blood cells were separated out from plasma by centrifugation at 6000 rpm for 15 min at 4°C (Eppendorf Centrifuge 5810R). Serum was immediately separated and stored in aliquots at -80°C until use.

Methodology

Detection of lipids, lipoproteins, hsCRP and routine laboratory parameters

Lipids, lipoproteins, and routine laboratory parameters were obtained in serum after a 14h overnight fasting. Using routine laboratory parameters, the level of creatinine and lipid were measured on a Siemens analyzer (Germany). Clinical and routine laboratory parameters are presented in Table 1. Low density lipoprotein cholesterol (LDL-C) was calculated according to the Friedewald formula [5]. Non-HDL-C was calculated as total cholesterol (TC) minus HDL-C. Lipoproteins apoAI, total apoAII, apoAIInonB, apoB, hsCRP were determined with immunonephelometric methods, using the Health Care Diagnostic Product (Siemens GmbH, Germany) on a Dade Behring nephelometer BNII System (Germany). Total apoCIII and apoCIIInonB were measured by electroimmunodiffusion, according to Laurell, using a commercial kit (Sebia, USA), TRLs (apoB:CIII and apoB:AII) were separated as non-HDL lipoproteins by using anti-apoB antibodies [12-14].

Determination of a concentration of LPO, LCAT and PON1 and CETP activity

Serum LPO concentration was measured using Cayman's Lipid Hydroperoxide. The Assay Kit (LPO) measures the hydroperoxides directly, utilizing the redox reactions with ferrous ions. Hydroperoxides are highly unstable and react readily with ferrous ions to produce ferric ions. The resulting

Table 1. Clinical and routine laboratory parameters in the reference group and in post-renal transplant patients (Tx)

	Reference group n=15	apoAI≥150mg/dl n= 39	apoAI≤150mg/dl n=28	All Tx patients n=67
Age	44 (22-50)	44 (24-59)	48 (19-66)	45 (19-66)
Gender (male/female)	7M, 8F	16M, 17F	18M, 16F	34M, 33F
BMI (kg/m²)	21.0 (18.5-24.7)	25.1 (21.2-34.5)*	25.6 (19.9-37.2)*	25.1 (20.2-37.2)*
Creatinine (μmol/L)	74 (62-99)	97.2 (61.8-132.6)*	114.9 (61.9-159.1)*	106.1 (69.9-159.1)*
eGFR M (ml/min/1.73 m²)	120 (103-127)	86.0 (62.5-143.7)*	79.9 (60.2-131.3)*	82.2 (60.2-143.7)*
eGFR F (ml/min/1.73 m²)	108 (98-120)	73.1 (53.1-122.1)*	67.9 (51.2-111.64)*	69.9 (51.2-122.1)*

Values are expressed as median (min-max); * - p<0.05 vs. the reference group

ferric ions are detected through utilizing the thiocyanate ion as the chromogen. Serum LCAT assay kit (ELISA, Life Inc., Wuhan, China) was used. The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of human LCAT in serum. The microtiter plate provided in this kit had been pre-coated with an antibody specific to LCAT. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for LCAT. Next, Avidin (a tetrameric or dimeric biotin-binding protein) in conjunction with Horse Radish Peroxidase (HRP) was added to each well. Only those wells that contained LCAT, biotin-conjugated antibody and enzyme-conjugated Avidin exhibited a colour change. The enzyme-substrate reaction was terminated by addition of a sulphuric acid solution, and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 10 nm. The LCAT mass (U/L – 1 μmol/min/L = amount of enzyme that catalyses the reaction of 1 μmol of substrate per minute under standard conditions) activity in the samples was then determined by comparing the O.D. of the samples to the standard curve. The PON-1 activity was measured by the modified Fourlong's method as described previously [12], from the initial velocity of p-nitrophenol production at 37°C, and its increased absorbance at 405 nm. This was recorded by an autoanalyzer (Cobas-Mira Plus, Roche Diagnostica, Switzerland). Serum was added to a basal assay mixture containing 100 mM TRIS-HCL buffer (pH 8.5) with paraoxon, 1 mM $CaCl_2$. A PON-1 activity of 1 U/L was defined as 1 mol of p-nitrophenol hydrolyzed per minute. A serum CETP assay kit (BioVision Inc. CA, USA), using a donor molecule containing a fluorescent self-quenched neutral lipid was transferred to an acceptor molecule in the presence of CETP. CETP-mediated transfer of the fluorescent neutral lipid to the acceptor molecule results in fluorescence (Excitation: 465 nm; Emission: 535 nm).

Statistical analysis

The data were expressed as medians and minimum-maximum. Statistical analysis of the results was performed using the non-parametric Kruskal-Wallis (KW) test for comparison of Tx patient groups and the reference group. The relation between hsCRP or LPO or LCAT or PON1 or CETP and concentration of lipid, lipoprotein, and lipoprotein ratios were examined by Spearman's correlation analysis. In the model of multiple regression analysis, high correlations between predictor variables result in inadequate regression coefficients. In such cases, multiple ridge stepwise forward regression analysis improves the accuracy of the model. In the model of multiple ridge forward stepwise regression analysis, hsCRP, LPO and PON1, LCAT, CETP were selected as the dependent variable and lipoproteins as the non-dependent variable, and for each of the non-dependent variables, parameters were calculated according to the equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n .$$

The relationship between the dependent variables is expressed by the coefficient of multiple regression (β), which provides information about the relationship between the dependent variables: hsCRP, LPO, PON1, LCAT, CETP, and lipids, lipoproteins and lipoprotein ratios as the non-dependents. The statistical significance of all variables was established at $p < 0.05$, and statistical analysis was performed using the STATISTICA software (StatSoft, Krakow, Poland).

RESULTS

The KW test (Table 2) clearly indicates that the Tx patients had moderate dyslipidemia and dyslipoproteinemia and slightly increased hsCRP, LPO, apoB:AII, apoCIII levels, but decreased LCAT mass, PON1 activity and lipoprotein ratios. Tx patients with apoAI < 150 mg/dl also had lower concentration of HDL-C, apoAI and lipoprotein ratios, and higher apoB:CIII levels than did Tx patients with apoAI > 150 mg/dl, but there were no difference in CETP activity between all groups. However, to make clear more relevant and essential correlations, multiple ridge stepwise forward regression was performed. The essential correlation coefficients are revealed in Table 3. The Spearman's correlations analysis were presented in Table 4. An analysis of these regression results in Tx patients with apoAI > 150mg/dl showed that concentration of hsCRP ($R^2 = 0.286$) positively correlated with total apoCIII ($\beta = 0.523$, $P = 0.007$) concentration. Moreover, a significant positive relationship was observed between PON1 activity ($R^2 = 0.452$) and apoAI ($\beta = 0.685$, $P = 0.001$) concentration, and between LCAT mass ($R^2 = 0.480$) and apoCIIInonB ($\beta = 0.535$, $P = 0.003$) and LDL-C ($\beta = 0.358$, $P = 0.038$) concentrations. Furthermore, CETP activity ($R^2 = 0.492$) was negatively correlated with apoB:CIII ($\beta = -0.540$, $P = 0.003$) and apoAI/apoB ($\beta = -0.580$, $P = 0.003$) ratios. In Tx patients with apoAI < 150 mg/dl, a significant negative correlation was noted between hsCRP level ($R^2 = 0.547$) and apoAI/apoB ($\beta = -0.378$, P=0.049) and apoAI/apoCIII ($\beta = -0.424$, $P = 0.049$) ratios. In addition, LPO concentration ($R^2 = 0.601$) was significantly positively correlated with apoB:AII ($\beta = 0.395$, $P = 0.038$) concentration. What is more, PON1 activity ($R^2 = 0.454$) significantly positively correlated with apoAI/apoB ratio ($\beta = 0.513$, $P = 0.044$), and a positive relationship was observed between LCAT mass ($R^2 = 0.610$) and apoB concentration ($\beta = 0.887$, $P = 0.00004$), and between LCAT mass and PON1 activity ($\beta = 0.242$, $P = 0.019$), as well as between LCAT mass and nonHDL-C ($\beta = 0.407$, P= 0.049) concentration. In the group of all Tx patients, hsCRP concentration ($R^2 = 0.203$) was positively correlated with total apoCIII concentration ($\beta = 0.557$, $P = 0.031$), and a positive correlation was found between LPO concentration ($R^2 = 0.332$) and apoB:AII concentration ($\beta = 0.369$, $P = 0.005$), PON1 activity ($R^2 = 0.276$) and apoAI concentration ($\beta = 0.359$, $P = 0.01$), LCAT mass ($R^2 = 0.356$) and apoB ($\beta = 0.403$, P=0.003) and apoCIIInonB concentrations ($\beta = 0.317$, $P = 0.017$), while CETP activity ($R^2 = 0.142$) negatively correlated with apoAI/apoB ratio ($\beta = -0.371$, $P = 0.018$).

Table 2. Concentration of lipid and lipoprotein, hsCRP, LPO, LCAT and lipid and lipoprotein ratios, as well as PON-1 and CETP activity in the reference group and in post-renal transplant patients (Tx) with apoAI>150mg/dl and with apoAI<150mg/dl and in all Tx patients (combined)

	Reference group n=15	apoAI>150mg/dl n=39	apoAI<150mg/dl n=28	All Tx patients n=67
TG (mmol/L)	0.99 (0.45-0.87)	1.36 (0.73-4.05)*	1.51 (1.02-3.29)**	1.38 (0.73-4.05)**
TC (mmol/L)	4.62 (3.55-4.84)	5.44 (3.60-8.47)*	4.92 (3.11-6.83)*	5.15 (3.11-8.47)*
LDL-C (mmol/L)	2.23 (1.19-2.60)	3.39 (2.02-4.81)**	3.23 (1.71-5.08)*	3.29 (1.71-5.08)**
HDL-C (mmol/L)	1.78 (1.56-2.02)	1.50 (1.06-1.94)	0.96 (0.69-1.11)***†	1.29 (0.69-1.94)***
nHDL-C (mmol/L)	2.82 (1.56-2.05)	4.04 (2.43-6.81)***	3.81 (2.41-5.80)***	3.86 (2.41-6.81)***
apoAI (mg/L)	1690 (1540-2660)	1760 (1530-2110)	1390 (930-1500)***†	1560 (930-2110)*
apoB (mg/L)	690 (550-670)	880 (510-1320)*	933 (697-1558)*	890 (510-1550)*
apoAII (mg/L)	292 (246-354)	345 (262-497)	297 (178-426)	326 (178-497)
apoAIInB (mg/L)	288 (28.2-341)	276 (181-396)	242 (170-332)	255 (170-396)
apoB:AII (mg/L)	4 (2-52)	69 (2-164)**	57 (2-157)**	66 (2-157)**
hCRP (mg/L)	0.26 (0.16-0.20)	1.44 (0.16-5.22)***	1.35 (0.16-10.97)***	1.37 (0.16-5.2)***
apoCIII (mg/L)	28 (27-41)	44 (18-74)*	45 (27-69)*	44 (18-74)*
apoCIIInB (mg/L)	20 (23-36)	33 (14-59)	29 (15-59)	31 (14-59)
apoB:CIII (mg/L)	8.0 (2.5-8.0)	11 (1.0-28.0)	16.0 (2.5-15.0)*	13.0 (1.0-28)*
LCAT (U/L)	360 (66-450)	205 (80-480)*	220 (92-460)*	210 (80-460)*
CETP (pmol/µl/hr)	48 (47-52)	48 (43-54)	48 (41-64)	48 (41-64)
PON-1 (mU/L)	268 (82-531)	133 (68-419)**	107 (55-333)**	113 (55-419)**
LPO µMol	0.435 (0.27-0.78)	1.51 (0.69-2.37)***	1.68 (0.93-2.52)***	1.59 (0.69-2.52)***
TC/HDL-C	2.54 (1.79-2.67)	3.79 (2.36-6.23)***	5.05 (3.48-7.13)***†	4.10 (2.36-7.13)***
LDL-C/HDL-C	1.24 (0.612-1.47)	2.40 (1.11-4.57)***	3.07 (2.18-5.21)***†	2.60 (1.11-5.20)***
TG/HDL-C	1.26 (0.52-1.08)	2.18 (1.11-5.61)***	3.53 (2.13-10.39)***†	2.56 (1.11-10.39)***
HDL-C/apoAI	0.40 (0.28-0.44)	0.33 (0.26-0.42)***	0.26 (0.18-0.37)***†	0.307 (0.18-0.47)***
apoAI/apoB	2.45 (2.25-3.90)	1.95 (1.35-3.36)**	1.51 (0.77-2.06)**	1.79 (0.77-3.36)**
apoAI/apoCIII	57 (42-62)	39 (28-64)**	34 (16-54)**	37 (16-64)**

Values are expressed as median (min-max); * - P<0.05; ** - P<0.01; *** - P<0.001 vs. the reference group;
† - P<0.05 vs. the Tx patients with apoAI>150mg/dl

Table 3. Multiple ridge forward regression between concentration of lipids, lipoproteins, and hsCRP, LPO, LCAT level and PON1 and CETP activity in all Tx patients, in Tx patients with apoAI>150mg/dl and apoAI<150mg/dl

	All Tx patients					Tx patients with apoAI>150mg/dl					Tx patients with apoAI<150mg/dl				
	hsCRP R^2=0.203 β	LPO R^2=0.332 β	PON1 R^2=0.276	LCAT R^2=0.356 β	CETP R^2=0.142 β	hsCRP R^2=0.286 β	LPO R^2=0 β	PON1 0.452 β	LCAT R^2=0.480 β	CETP R^2=0.492 β	hsCRP R^2= 547 β	LPO R^2=0.601 β	PON1 R^2 =0.454 β	LCAT R^2= 0.610 β	CETP R^2=0 β
LDL-C									0.358*						
nonHDL-C														0.407*	
apoAI			0.359**					0.685***							
apoAII															
apoB:AII		0.369**										0.395*			
apoCIII	0.557*					0.523**									
apoB:CIII										-0.540**					
apoCIIInB				0.317**					0.535**						
apoB				0.403**										0.887***	
PON1														0.242*	
apoAI/apoB					-0.371*					-0.580**	-0.378*		0.513*		
apoAI/ apoCIII											-0.424*				

* - p < 0.05; **- p < 0.01; *** - p < 0.001

DISCUSSION

Cardiovascular diseases are major causes of mortality of renal transplant (RT) recipients [17]. Reverse kidney renal failure after renal transplantation is associated with various types of metabolic dysfunctions [2], and immunosuppressive therapy seems to be the main factor that influences the post-transplant lipidemic profile [19]. Our previously

Table 4. Spearman's correlation between hsCRP, LPO, LCAT, PON1, CETP and lipid lipoprotein and lipoprotein ratios in Tx patients with apoAI≥150mg/dl and apoAI≤150mg/dl and in all Tx patients

	Tx patients														
	hsCRP R mg/dl			LPO R m			PON-1 R			LCAT R			CETP R		
	≥150 mg/dl n=39	≤150 mg/dl n=28	ALL mg/dl n=67	≥150 mg/dl n=39	≤150 mg/dl n=28	ALL mg/dl n=67	≥150 mg/dl n=39	≤150 mg/dl n=28	ALL mg/dl n=67	≥150 mg/dl n=39	≤150 mg/dl n=28	ALL mg/dl n=67	≥150 mg/dl n=39	≤150 mg/dl n=28	ALL mg/dl n=67
TG	0.001	-0.065	-0.006	-0.044	0.120	0.021	-0.149	0.153	-0.053	-0.086	-0.127	-0.121	-0.164	0.045	-0.025
TC	0.262	-0.120	0.101	-0.292	-0.069	-0.230	0.070	0.021	0.100	0.199	-0.275	-0.024	-0.056	-0.063	-0.081
LDL-C	0.297	-0.009	0.148	-0271	-0.058	-0.195	0.079	-0.083	0.081	0.235	-0.256	0.031	-0.028	-0.048	-0.069
HDL-C	0.065	-0.351	-0.032	-0.023	-0.048	-0.142	0.038	0.337	0.228	-0.165	-0.369[1]	-0.187	-0.047	-0.131	-0.110
nHDL-C	0.278	-0.036	0.128	-0.301	-0.033	-0.175	0.061	-0.025	0.078	0.195	-0.254	-0.001	-0.021	-0.045	-0.076
apoAI	-0.006	-0.391[1]	-0.070	0.142	0.079	-0.057	0.425[1]	0.489[2]	0.414[3]	-0.011	-0.128	0.045	-0.235	-0.150	-0.195
apoAII	-0.042	-0.143	-0.072	0.165	0.243	0.128	0.167	0.471[1]	0.388[2]	0.253	-0.046	0.078	-0.110	0.068	-0.92
apoAIInB	-0.084	-0.238	-0.133	0.07	-0.201	-0.077	0.086	0.270	0.261[1]	0.228	0.187	0.165	0.124	-0.147	-0.58
apoB:AII	0.076	-0.100	0.003	0.184	0.458[1]	0.300[1]	0.123	0.424[1]	-0.190	0.032	-0.223	-0.065	-0.209	0.160	-0.068
apoB	0.28	0.558[2]	0.336[2]	-0.332[1]	-0.083	-0.210	0.196	-0.234	-0.006	0.345[1]	0.337	0.309[1]	0.118	0.126	0.146
apoCIII	0.492[2]	0.431[1]	0.468[3]	-0.387[1]	-0.343	-0.347[2]	-0.027	-0.079	-0.046	0.443[2]	0.349	0.443[3]	-0.104	0.055	-0.040
apoCIIInB	0.363[1]	0.397[1]	0.378[2]	-0.345[1]	-0.236	-0.271[1]	0.088	-0.61	0.023	0.396[1]	0.395[1]	0.416[3]	0.067	0.102	0.090
apoB:CIII	0.282	0.348[1]	0.268[1]	0.031	-0.235	-0.097	-0.111	-0.284	-0.114	0.087	0.303	0.129	-0.303	-0.008	-0.205
HDLc/AI	0.193	-0.011	0.098	-0.126	-0.272	-0.222	-0.207	0.004	-0.084	-0.162	-0.331	-0.214	0.132	0.001	0.071
apoAI/ apoB	-0.175	-0.627[3]	-0.286[1]	0.338[1]	0.208	0.180	-0.031	0.379[1]	0.234	-0.327[1]	-0.366[1]	-0.295[1]	-0.165	-0.206	-0.240
apoAI/ CIII	-0.486[2]	-0.517[2]	-0.454[3]	0.390[1]	0.412[1]	0.324[1]	0.153	0.200	0.224	-0.443[2]	-0.301	-0.389[2]	0.055	-0.145	-0.075

1 – p=0.05; 2 – p=0.01; 3 – p=0.001, apoAI/CIII = apoAI/apoCIII

results indicated that, in such a case, VLDL, IDL, LDL and HDL particles were smaller, dense and more susceptible to modification and oxidation. Furthermore, they were exposed to oxidative stress and the anti-oxidative role PON-1 was weakened [12-14]. However, the stepwise switch from CSA to MyFortic was safe and mostly successful. Moreover, it had beneficial effects on blood pressure, glomerular haemodynamics and lipid profiles [12-14].

The results of the presented study showed that the increased concentrations of apoCIIInonB and apoCIII induced an inflammatory state in blood vessels, and the increase in hsCRP level, as well as the remodeling of the HDL and VLDL particles generated an increase in LPO level. At the same time, apoAI induced an increase of PON1 activity, whereas apoCIIInonB and LDL-C level increased LCAT mass, and the changed apoAI/apoB ratio brought about a decrease of CETP activity in Tx patients with higher apoAI level. Moreover, Tx patients with lower apoAI and HDL-C level showed that concentration of hsCRP was decreased by raised apoAI/apoB and apoAI/apoCIII ratios, while at the same time, apoB:AII increased LPO level. Furthermore, the changed apoAI/apoB ratio increased PON1 activity, and the increased concentrations of apoB, nonHDL-C and PON1 activity also enhanced LCAT mass. However, these results suggest considerable remodeling in the composition of both HDL and VLDL particles. We hold that the higher levels of lipids, and total apoCIII, TRLs (apoB:CIII, apoB:AII) disturbed lipid and lipoprotein particles composition. In addition, the decrease in apoAI level and the increase in apoB and apoCIII concentrations may contribute to increased nonHDL-C level, and aggravated renal graft, accelerated atherosclerosis and chronic heart diseases [12-14].

The results of the presented studies and those by of other laboratories have already shown that TG-rich apoB-containing apoCIII lipoproteins are linked to inflammation [1,2,12-14], and that oxidation is not always proatherogenic [7,15]. Moderate oxidation that encompasses *in vivo* conditions destabilizes VLDL and promotes the fission of HDL-size particles. Consequently, mild oxidation may be synergistic with the lipoprotein lipase reaction, and, hence, may help to accelerate VLDL metabolism. The most physiologically relevant species is the "minimally oxidized" lipoprotein that carries lipid peroxides and their products, but has minimal protein modifications [7]. Our work and that of others show that plasma apoAI concentration may influence HDL subclass profile more significantly than do the plasma lipids concentrations. ApoAII, the second-most abundant apo in HDL particles, appears to play a complex role in the metabolism of HDL subclasses and could have important antiatherogenic properties, such as the maintenance of a stable HDL pool and the accumulation of small-sized HDL_3. ApoAI/apoB ratio could, therefore, reliably and sensitively reflect the HDL subclass profile [20]. Recently, Hine *et al*. [9] reported the results of *in vitro* studies. They concluded that ApoAI, LCAT, and PON1 have separate antioxidant activity in preventing the LDL, ApoAI, LCAT and PON1 oxidation, and, moreover, all collectively contributed to the HDL antioxidant activity. The combination of apoAI, LCAT, and PON1 increase the time in that HDL is capable of preventing LDL oxidation. During this time, LCAT oxidative inactivation is retarded. Kar *et al*. [11] showed that increased (Ox-PL) content: (a) modifies the physicochemical properties of the lipid domain of the rHDL particles; (b) decreases the stability and alters the conformation, as well as orientation of apoAI molecules on the rHDL particles;

and (c) decreases the PON1stimulation capacity of the rHDL particles. They concluded that the presence of Ox-PLs destabilizes the structure of the HDL particles and modifies their function. In addition, other researchers have reported that atherogenic HDL dysfunction and impaired reverse cholesterol transport occur in human inflammatory syndromes, independent of significant change in plasma HDL-C levels, and suggest that experimental in vitro human inflammation induces HDL remodeling and loss of HDL atheroprotective functions in a model that is broadly relevant to diverse human inflammatory disorders [4].

Limitation of the Study

This study has several limitations. Additional studies are needed for a larger reference group. What is more, proteinuria and higher concentration of creatinine in Tx patients can confound our results. In addition, more than half of the cohort of our Tx patients took statins, hence, a large cohort of patients should be divided into two groups: one with statins therapy and the other without statins therapy.

CONCLUSIONS

The results of the presented study in Tx patients who received immunosuppressive therapy without acute inflammatory diseases, show for the first time that higher apoAI/apoB and apoAI/apoCIII ratios induced a decrease of the hsCRP concentration, while the composition of apoCIII-nonB, LDL-C and apoAI brought about an increase of LCAT mass and PON1 activity. Conversely, in Tx patients with lower apoAI level, the increased concentration of apoCIII aggravated the inflammatory state in the blood vessels and increased hsCRP level, while, at the same time, the increased concentration apoB:AII in VLDL enhanced the mild oxidation of lipoprotein, and it elevated the concentration of LPO. Furthermore, apoAI/apoB composition increased PON1 activity, as well as apoB and nonHDL-C levels, respectively, while enhanced PON1 activity increased LCAT mass. We noted that the structure of cholesterol- and triglycerides-carrying particles is highly connected with the composition and concentration of the determined molecules, e.g. hsCRP, LPO, apolipoproteins such as apoAI, apoAII and TRLs, as well as LCAT, PON1 and CETP, and their lipid and lipoprotein ratios. Changes in these parameters can induce a rearranging of the HDL particle, and, simultaneously, a remodeling of the VLDL particle. This may be beneficial for antioxidant activity, and it might reverse cholesterol transport in Tx patients with higher apoAI levels, but may be atherogenic in those with lower apoAI, and could accelerate the rejection of the transplant and induce cardiovascular diseases. Recognition of those relationships may be useful in the explanation of some synergism in the self-curing action of some lipoproteins, as well as in development of curing methods of aggravated renal graft rejection, accelerated atherosclerosis and chronic heart disease. Such metabolic pathways can be used as potentially novel targets for pharmacological intervention. However, more research is needed to fully understand this problem.

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