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Characteristics of low-density and high-density lipoprotein subclasses in pediatric renal transplant recipients

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Summary

Renal transplant recipients often suffer from dyslipidemia which is one of the principal risk factors for cardiovascular disease. This study sought to determine characteristics of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) particles and their associations with carotid intima-media thickness (cIMT) in a group of pediatric renal transplant recipients. We also examined the influence of immunosuppressive therapy on measured LDL and HDL particle characteristics. HDL size and subclass distribution were determined using gradient gel electrophoresis, while concentrations of small, dense LDL (sdLDL)-cholesterol (sdLDL-C) and sdLDL-apolipoprotein B (sdLDL-apoB) using heparin-magnesium precipitation method in 21 renal transplant recipients and 32 controls. Renal transplant recipients had less HDL 2b ($P < 0.001$), but more HDL 3a ($P < 0.01$) and 3b ($P < 0.001$) subclasses. They also had increased sdLDL-C ($P < 0.01$) and sdLDL-apoB ($P < 0.05$) levels. The proportion of the HDL 3b subclasses was a significant predictor of increased cIMT ($P < 0.05$). Patients treated with cyclosporine had significantly higher sdLDL-C and sdLDL-apoB concentrations ($P < 0.05$) when compared with those on tacrolimus therapy. Pediatric renal transplant recipients have impaired distribution of HDL and LDL particles. Changes in the proportion of small-sized HDL particles are significantly associated with cIMT. Advanced lipid testing might be useful in evaluating the effects of immunosuppressive therapy.

Introduction

Although renal transplantation represents a choice treatment for patients with end-stage renal disease (ESRD), long-term survival of pediatric renal transplant recipients is still lower than in the general population [1]. Renal transplant recipients, in common with other patients with varying degrees of renal failure, have an increased risk of cardiovascular disease (CVD) development [2]. Abnormalities in lipid homeostasis, as the main risk factors for CVD, are frequently seen in such category of patients [3]. Numerous studies have demonstrated that the quality of high-density lipoprotein (HDL) and low-density lipoprotein (LDL) particles, rather than simply the levels of HDL-cholesterol (HDL-C) and LDL-cholesterol (LDL-C), exert a potent influence on CVD risk [4,5]. Indeed, both small, dense LDL (sdLDL) and small-sized HDL particles have emerged as novel CVD risk factors [6]. So far, heterogeneity of LDL and HDL particles has been studied less extensively in pediatric renal transplant recipients.

Early atherosclerotic lesions in asymptomatic individuals are commonly assessed by measuring the intima-media thickness (IMT) of the affected vessel. This method is widely used for carotid arteries and increased carotid

IMT (cIMT) predicts the risk of developing myocardial infarction and stroke in the general population [7], as well as in ESRD patients [8]. In addition, studies in the pediatric population have revealed significant associations between cIMT and traditional CVD risk factors [9]. However, the possible contribution of sdLDL and small-sized HDL, beyond traditional risk factors, to increased cIMT in renal transplant recipients remains to be elucidated.

Significant sources of post-transplant dyslipidemia in both adults and children are side effects of immunosuppressive medications. It has been shown that cyclosporine administration is more likely associated with dyslipidemia than the use of tacrolimus [10]. However, the impact of particular immunosuppressive drugs on HDL and LDL particle characteristics has been scarcely investigated.

The aim of this study was to evaluate HDL and LDL particle characteristics in a group of pediatric renal transplant recipients. In addition, we have examined their relationship with cIMT and have evaluated the effects of cyclosporine and tacrolimus immunosuppressive therapy on measured LDL and HDL particle characteristics.

Patients and methods

Subjects

The study group consisted of 21 adolescent patients who underwent renal transplantation during childhood (13 male and eight female; mean age 15.00 ± 3.21 years; range 9–21). All the patients were recruited in the Nephrology Department at the University Children's Hospital in Belgrade, where they were treated. Underlying renal diseases included: congenital abnormalities of the kidney and urinary tract ($n = 11$), polycystic kidney disease ($n = 4$), Jeune's syndrome ($n = 1$), irreversible tubular necrosis ($n = 1$), Joubert syndrome ($n = 1$), focal segmental glomerulosclerosis ($n = 1$), hemolytic uremic syndrome ($n = 1$), and diffuse mesangial sclerosis ($n = 1$). Exclusion criteria included primary cardiac disease, diabetes mellitus and any co-morbid condition, such as infectious disease or acute transplant rejection at the time of enrollment. At the point of study entry, height and weight [for body mass index (BMI) calculation] and blood pressure were measured. Clinical characteristics of our study group are listed in Table 1.

Regarding current medical treatment, 17 patients were receiving triple immunosuppressant therapy (prednisone/tacrolimus/mycophenolate mofetil, $n = 7$; prednisone/cyclosporine/mycophenolate mofetil, $n = 5$; prednisone/sirolimus/mycophenolate mofetil, $n = 1$; prednisone/cyclosporine/azathioprine, $n = 3$; prednisone/tacrolimus/azathioprine, $n = 1$). One patient was on prednisone/cyclosporine combination therapy and three patients received quadruple immunosuppressant therapy (prednisone/sirolimus/tacrolimus/mycophenolate mofetil). Sixteen patients were receiving antihypertensive therapy, while no subjects received hypolipemic agents. The control group was selected from a preliminary pool of 124 healthy adolescents (59 males and 65 females, aged from 11 to 18 years) who were recruited in various health centers in Belgrade during regular annual medical check-ups and were free of any acute or chronic pathological condition. The final selection of the control group included in this study was accomplished by matching: age, male/female proportion and BMI values with the patients. As a result, 32 age-, gender- and BMI-matched healthy normotensive adolescents formed the control group. None of the subjects in this group used any prescribed medications. Both patients and controls were Serbian Caucasians. Informed consent was obtained from each subject enrolled in the study, or in the case of minors, from a parent or tutor. The whole study was planned and executed following the ethical guidelines of the Helsinki Declaration. According to institutional guidelines, the Ethics Committee at the Faculty of Pharmacy, University of Belgrade approved the whole study protocol.

Table 1. Clinical characteristics of renal transplant recipients.

	Renal transplant recipients; $n = 21$
Age (years)	15.00 ± 3.21
Gender, male (%)	61.9
BMI (kg/m^2)	20.23 ± 3.58
Systolic blood pressure (mmHg)	119.25 ± 12.06
Diastolic blood pressure (mmHg)	76.50 ± 8.90
cIMT (mm)	0.41 ± 0.09
Duration of chronic kidney disease (years)	11.05 ± 5.61
Hemodialysis prior transplantation (%)	77.3
Hemodialysis duration (months)	4.44 ± 3.32
Post-transplantation interval (years)*	4 (1–5)
Creatinine clearance (ml/min)	84.65 ± 32.56

BMI, body mass index; cIMT, carotid intima-media thickness.
Continuous variables are presented as mean ± standard deviation, whereas categorical variables are presented as relative frequencies.
*Numeric value is presented as median and interquartile range.

Carotid intima-media thickness (cIMT) measurement

A single sonographer who was specifically trained to carry out the prescribed study examination performed the IMT measurement. The sonographer was uninformed and unfamiliar with the patient's diagnosis, therapy and laboratory findings. All the measurements were achieved in a standard fashion with patients in the supine position and their head turned slightly to the contralateral side. Images were taken from longitudinal views of the far wall of left and right common carotid arteries, bifurcation and inter-

nal carotid arteries at the bulb level. Each artery was analyzed from proximal to distal and a measurement was performed 10 mm below the carotid bifurcation in common carotid arteries, at the bifurcation level and 10 mm after the carotid bifurcation in the internal carotid arteries. IMT was defined as the distance from the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall. The IMT represents the mean values of both sides. All the results were achieved on a single ultrasound machine Siemens ACUSON Antares, using a linear array 7 MHz scan head with standardized image settings, including resolution mode, depth of field, gain and transmission focus. The measurements were performed semi-automatically using a software enhancement for IMT measurement (AHP-Arterial Health Package Siemens Software). After placing the ECG electrodes and starting the program recordings were triggered at the end of the diastolic cycle. Following image acquisition and starting the calculating option, IMT was calculated and printed. The data were archived on hard disk until the end of the study. The overall coefficient of variation for repeated measures of cIMT was <4%.

Biochemical analyses

Blood samples were collected into evacuated tubes containing EDTA and serum sample tubes after a 12-hour fasting period. Plasma and serum were separated by immediate centrifugation at 1500 × *g* for 10 min at 4 °C. Aliquots of each sample were stored at −80 °C. The samples were thawed immediately before analyses.

Serum urea, creatinine, total protein, albumin, and hemoglobin concentrations were measured by routine laboratory methods. Glomerular filtration rate was assessed by measuring 24-h creatinine clearance. Serum total cholesterol (TC), free cholesterol (FC) and triglyceride (TG) concentrations were assayed using enzymatic methods using an ILab 600 analyzer (Instrumentation Laboratory, Lexington, MA, USA). The concentration of HDL-C was measured using the same enzymatic method after precipitation of the plasma with phosphotungstic acid in the presence of magnesium ions. The concentration of LDL-C was determined using a direct homogeneous assay (Olympus Diagnostica GmbH, Hamburg, Germany). Apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB) were measured using immunoturbidimetric assays (Dialab, Vienna, Austria).

HDL particle size and subclass determination

Plasma HDL particles were separated using a method previously described by Rainwater *et al.* [11]. Composite (3–31%) polyacrylamide gradient gels were used for electrophoretic separations of HDL subfractions. A detailed description of the procedure has been published elsewhere [12,13]. In brief, electrophoresis was performed at 8 °C in a Hoefer SE 600 Ruby electrophoresis unit (Amersham Pharmacia Biotech, Vienna, Austria) using Tris (90 mM)-boric acid (80 mM)-Na_2EDTA (2.7 mM) buffer, pH 8.35 for 20 h. Gels were calibrated using the Pharmacia High Molecular Weight protein standards and carboxylated polystyrene microsphere beads. After electrophoretic separation, the gels were stained for proteins with Coomassie brilliant blue G-250 and for lipids with Sudan black. Gels were analyzed using Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria) with Image Quant software (version 5.2;1999; Molecular Dynamics, Sunnyvale, CA, USA). The migration distance for each absorbance peak was determined and the particle diameter corresponding to each peak was calculated from the calibration curve. The estimated diameter of the major peak in HDL region of each scan was referred to as the dominant particle diameter. The relative content of each HDL subclass was estimated by determining the areas under the peaks of densitometric scan of the sample, as previously reported [12,13].

LDL particle characterization

Low-density lipoprotein particles characterization was achieved by measuring concentrations of cholesterol and apoB within the sdLDL fraction (sdLDL-C and sdLDL-apoB, respectively) using the heparin-magnesium precipitation method [14,15]. In brief, a serum sample (150 μl) was mixed with precipitation reagent (150 μl) containing 150 U/ml of heparin sodium salt and 90 mmol/l $MgCl_2$, incubated for 10 min at 37 °C, placed in an ice bath for 15 min and centrifuged at 15000 rpm for 15 min at 4 °C. An aliquot of the supernatant was used to measure LDL-C and apoB concentrations. The proportions of sdLDL-C and sdLDL-apoB in total LDL-C and LDL-apoB, respectively, were calculated as (sdLDL-C/serum LDL-C) × 100 and (sdLDL-apoB/serum apoB) × 100 and expressed in percents.

Lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities measurement

Lecithin:cholesterol acyltransferase (LCAT) and cholesteryl ester transfer protein (CETP) activities were determined by following a previously described method [16–18]. In principle, LCAT activity was determined as a function of the decrease in the amount of FC in plasma after 3 h of incubation at 37 °C. CETP activity was assessed as the rate of cholesterol esters (CE) transfer from HDL to VLDL and LDL as a function of time. CE mass transfer was measured as the difference between the

rate of decrease in FC in the whole plasma and the rate of increase of CE in HDL during 3 h of incubation at 37 °C. Accordingly, molar LCAT activity was calculated as initial plasma FC − final plasma FC, while CETP activity as: (initial plasma FC − final plasma FC) − [(final HDL-TC − final HDL-FC) − (initial HDL-TC − initial HDL-FC)]. All assays were performed in duplicate.

Statistical analysis

Differences in continuous variables between the groups were analyzed using the Student's *t*-test for normally distributed variables. As the distributions of TG, LCAT, CETP, sdLDL-C, sdLDL-apoB, % sdLDL-C, and % sdLDL-apoB were skewed, the data are presented as median (interquartile range) and compared using the Mann–Whitney *U*-test. Group differences for categorical variables were examined using the chi-squared test. Univariate associations were evaluated using Spearman's correlation analysis. Subsequent stepwise multiple regression analysis was used to estimate the contribution of independent predictors to the variance in cIMT. Differences with $P < 0.05$ were considered to be statistically significant. All calculations were performed using MedCalc software.

Results

To determine differences in serum lipids and lipoproteins, we compared the renal transplant recipients group with an age-, gender-, and BMI-matched control group of healthy children. The patients had significantly higher TC, TG, and apoB concentrations. Plasma CETP activity was significantly higher in patients than in controls. No differences in LCAT activity were seen between the two groups. Regarding differences in renal function parameters, the concentrations of creatinine and urea were higher and the concentration of albumin was lower in the patients (Table 2).

To gain further insight into the characteristics of lipoprotein particles, we analyzed HDL subclass distributions in patients and controls (Fig. 1). Our results indicated that patients had a significantly lower relative proportion of the large HDL 2b subclass in addition to a significant increase in the proportions of small HDL 3a and 3b subclasses.

The differences between patients and controls in sdLDL-C, sdLDL-apoB, sdLDL-C (%), and sdLDL-apoB (%) are shown in Fig. 2. Renal transplant recipients had higher concentrations of sdLDL-C and sdLDL-apoB, as well as higher sdLDL-C (%).

We next analyzed correlations between serum lipids, lipoproteins, and parameters of renal function with the measured characteristics of HDL and LDL particles in the patients (Table 3). Our results revealed significant positive correlation of HDL diameter with HDL-C, while negative correlation was found with TG concentration. The relative proportion of HDL 2b correlated positively, while the proportions of HDL 3a and 3b correlated negatively with HDL-C and apoA-I concentrations. Regarding the relationship with parameters of renal function and other recorded characteristics of patients, we found that the duration of chronic kidney disease correlated negatively with the proportion of HDL 2b and positively with the proportion of HDL 3a.

We also found significant positive correlations between TG concentration and sdLDL-C, sdLDL-apoB, and sdLDL-C (%). Serum apoB levels correlated positively with sdLDL-C. The measured characteristics of LDL particles showed no correlations with the examined parameters of renal functions.

Possible associations between different features of HDL and LDL particles were next examined. The results showed significant negative correlations of HDL diameter with sdLDL-C and sdLDL-C (%).

Table 2. Clinical characteristics, renal function parameters, and lipid and lipoprotein parameters in renal transplant recipients and control group.

	Renal transplant recipients; $n = 21$	Controls; $n = 32$	P
Age (years)	15.00 ± 3.21	14.06 ± 1.25	0.140
Gender, male (%)	61.9	59.4	0.854
BMI (kg/m^2)	20.23 ± 3.58	20.21 ± 4.09	0.986
Systolic blood pressure (mmHg)	119.25 ± 12.06	114.22 ± 9.85	0.107
Diastolic blood pressure (mmHg)	76.50 ± 8.90	73.44 ± 6.89	0.192
Hemoglobin (g/l)	124.53 ± 15.82	130.66 ± 11.11	0.120
Creatinine (μmol/l)	95.43 ± 33.62	23.19 ± 5.46	<0.001
Urea (mmol/l)	7.51 ± 2.40	4.23 ± 1.74	<0.001
Total proteins (g/l)	68.48 ± 5.53	70.59 ± 4.83	0.146
Albumin (g/l)	44.50 ± 2.43	50.49 ± 5.86	<0.001
TC (mmol/l)	5.00 ± 0.99	4.46 ± 0.82	<0.05
LDL-C (mmol/)	2.87 ± 0.89	2.85 ± 0.81	0.943
HDL-C (mmol/l)	1.36 ± 0.51	1.30 ± 0.24	0.578
ApoA-I (g/l)	1.65 ± 0.42	1.75 ± 0.26	0.359
ApoB (g/l)	1.01 ± 0.26	0.84 ± 0.21	<0.01
TG (mmol/l)*	1.61 (0.84–2.27)	0.64 (0.50–0.77)	<0.001
Dominant HDL particle diameter (nm)	10.64 ± 0.78	10.76 ± 0.80	0.574
LCAT activity (μmol/l/h)*	24.5 (18.8–37.6)	19.6 (13.5–25.9)	0.110
CETP activity (μmol/l/h)*	27.4 (20.3–37.7)	15.9 (11.1–28.4)	<0.05

BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; ApoA-I, apolipoprotein A-I; ApoB, apolipoprotein B; TG, triglyceride; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein.
Variables are presented as mean ± standard deviation and compared using the Student's *t*-test.
*Values are presented as median (interquartile range) and compared using the Mann–Whitney *U*-test.

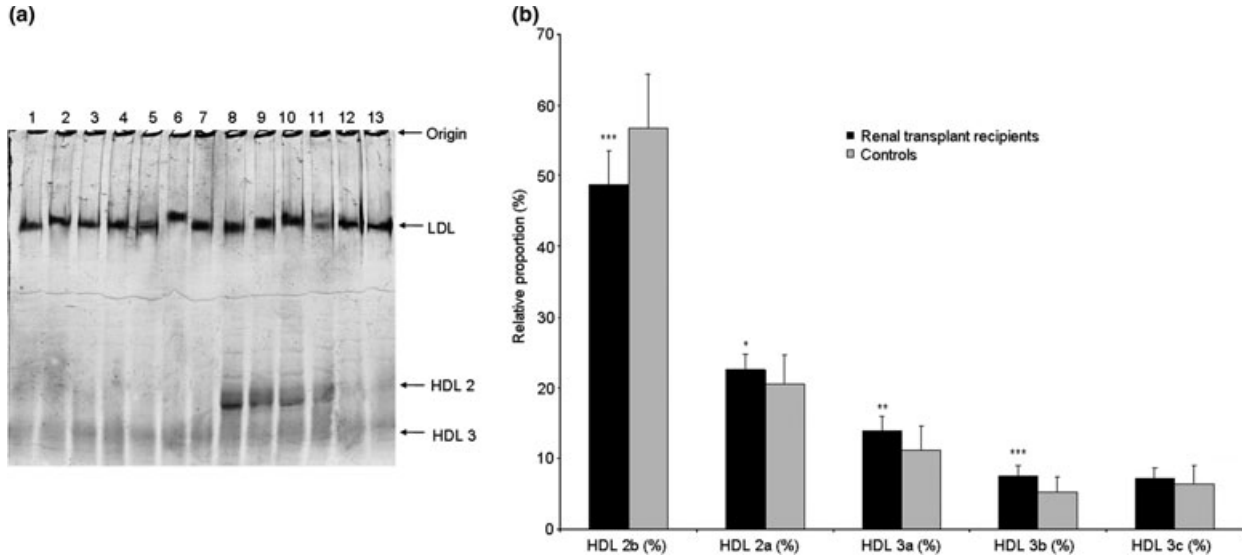

Figure 1 High-density lipoprotein subclass distribution in renal transplant recipients and controls. (a) Electrophoretic patterns of HDL subclasses (lanes 1–7: patients, lanes 8–13: controls); (b) Relative proportions of HDL subclasses in patients and controls. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. HDL, high-density lipoprotein.

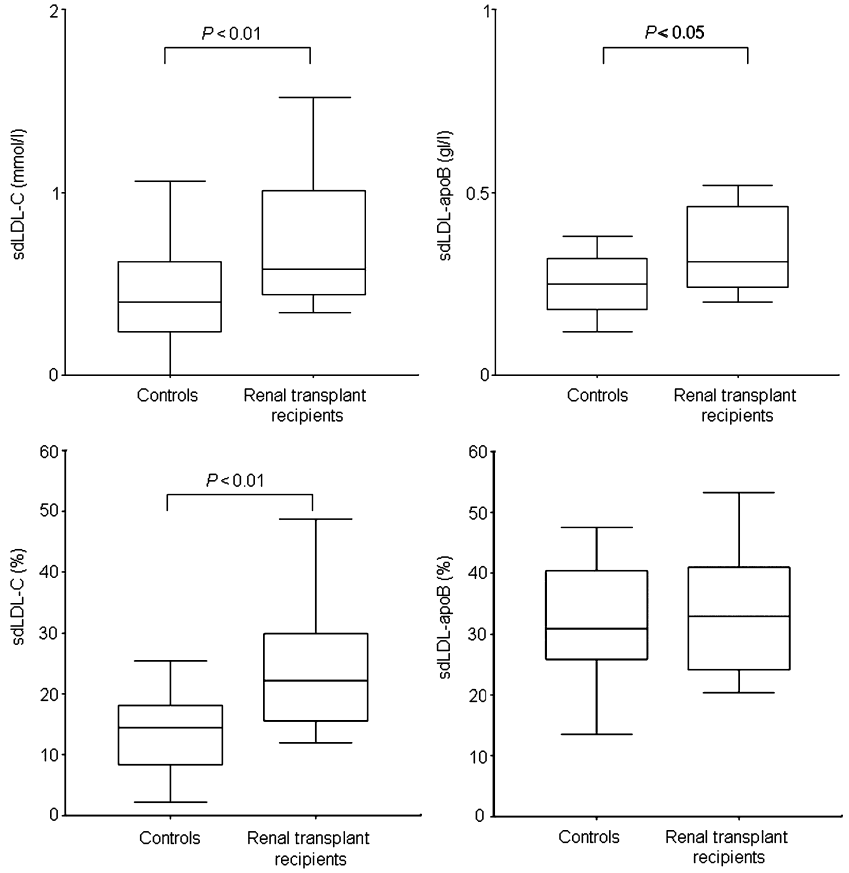

Figure 2 Concentrations of sdLDL-C and sdLDL-apoB in renal transplant recipients and controls. Lower and upper lines indicate the 10th and 90th percentiles. The box indicates the 25th and 75th percentiles, while the line in the box indicates the median value. sdLDL-C, small, dense low-density lipoprotein-cholesterol; sdLDL-apoB, small, dense low-density lipoprotein-apolipoprotein B.

As we aimed to explore the associations between both HDL and LDL particle characteristics with development of CVD in renal transplant recipients, we used cIMT as a marker of early, subclinical atherosclerosis. Univariate correlation analysis showed that cIMT correlated with age, concentration of TG, HDL diameter, and relative

Table 3. Correlations between serum lipids, lipoproteins, and parameters of renal function with the measured characteristics of HDL and LDL particles in renal transplant recipients.

Parameter	HDL diameter (nm)	HDL 2b (%)	HDL 2a (%)	HDL 3a (%)	HDL 3b (%)	HDL 3c (%)	sdLDL-C (mmol/l)	sdLDL-C (%)	sdLDL-apoB (g/l)	sdLDL-apoB (%)
HDL-C (mmol/l)	0.485*	0.617**	−0.257	−0.622**	−0.567**	−0.301	−0.373	−0.371	−0.370	−0.309
apoA-I (g/l)	0.350	0.441*	0.112	−0.494*	−0.621**	−0.212	−0.220	−0.419	−0.263	−0.330
ApoB (g/l)	−0.105	−0.232	−0.135	0.137	−0.009	0.040	0.633**	−0.058	0.419	−0.379
TG (mmol/l)	−0.574**	−0.291	0.176	0.406	0.238	0.102	0.733**	0.625**	0.607**	0.360
HDL diameter (nm)	–	0.477*	0.257	−0.478*	−0.254	0.054	−0.452*	−0.485*	−0.378	−0.350
Duration of chronic kidney disease (years)	−0.153	−0.537*	0.093	0.483*	0.350	0.296	0.405	0.242	0.343	0.037

HDL, high-density lipoprotein; sdLDL-C, small, dense low-density lipoprotein-cholesterol; sdLDL-apoB, small, dense low-density lipoprotein-apolipoprotein B; ApoA-I, apolipoprotein A-I; TG, triglyceride.
*$P < 0.05$; **$P < 0.01$.

Table 4. Univariate and stepwise multiple linear regression analysis for cIMT in renal transplant recipients.

Parameters		R		*P*	
Univariate analysis					
Age (years)		0.554		<0.05	
TG (mmol/l)		0.538		<0.05	
HDL diameter (nm)		−0.591		<0.05	
HDL 3b (%)		0.613		<0.05	
Model	Predictors	β (SE β)	*P*	Adjusted R^2 for model	
Multiple linear regression analysis					
Model 1*	HDL 3b (%)	0.600 (0.016)	<0.05	0.302	
Model 2†	HDL 3b (%)	1.092 (0.015)	<0.01	0.805	
	Systolic blood pressure (mm Hg)	0.649 (0.001)	<0.05		

cIMT, carotid intima-media thickness; TG, triglyceride; HDL, high-density lipoprotein.
*Variables included in the model were: age, gender (0-woman, 1-man), BMI, TC, HDL-C, LDL-C, TG, apoA-I, apoB, sdLDL-C, sdLDL-apoB, dominant HDL diameter, HDL subclasses.
†Variables included in the model were as follows: systolic blood pressure, diastolic blood pressure, duration of chronic kidney disease, post-transplantation interval, hemodialysis prior transplantation (0-no, 1-yes), creatinine clearance, serum creatinine concentration, serum urea concentration, hemoglobin concentration and all variables from Model 1.
Values are given as correlation coefficients (R) and β (SE β).

proportion of HDL 3b. To reveal significant and independent determinants of cIMT, we performed multiple linear regression analysis (Table 4). When age, gender, BMI, and examined lipid and lipoprotein parameters were included in Model 1, only the relative proportion of HDL 3b was a significant predictor of cIMT. After addition of renal disease markers, duration of chronic kidney disease, hemodialysis prior to transplantation, post-transplantation interval, and blood pressure in Model 2, the proportion of HDL 3b retained its significance.

Lastly, we analyzed the impact of administration of different immunosuppressants on HDL and LDL particle characteristics. Patients receiving cyclosporine therapy ($n = 9$) had a significantly higher sdLDL-C and sdLDL-apoB concentrations when compared with patients on tacrolimus therapy ($n = 12$) (Fig. 3). Similar unfavorable changes were also seen for other LDL and HDL particle characteristics. The relative proportion of HDL 2b was lower (46.9 ± 4.3% vs. 49.6 ± 4.9%), while the relative proportions of HDL 3a (14.4 ± 2.5% vs. 13.6 ± 1.9%), HDL 3b (7.7 ± 1.5% vs. 7.4 ± 1.5%), and HDL 3c (7.3 ± 1.1% vs. 7.1 ± 1.8%) were higher in patients treated with cyclosporine when compared with the tacrolimus-receiving patient group. However, none of the observed differences reached statistical significance.

Discussion

Dyslipidemia is recognized as one of the principal CVD risk factors in renal transplant recipients. In this study, we have observed a significant shift in HDL particle distribution toward smaller subclasses (Fig. 1) in pediatric renal transplant recipients, despite the fact that we found no differences in the dominant HDL diameter and HDL-C level between patients and controls (Table 2). Likewise, sdLDL-C and sdLDL-C (%) were higher in renal transplant recipients (Fig. 2), although serum LDL-C levels were not different when compared with controls. In the study by Siirtola *et al.* [19], unfavorable changes in serum levels of TC, LDL-C, and HDL-C were less prevalent and less severe in pediatric renal transplant recipients when compared with reports in adults. Correspondingly, we found that apart from TC, TG, and apoB concentrations, other traditional lipid measurements did not differ between our two examined groups (Table 2). Therefore,

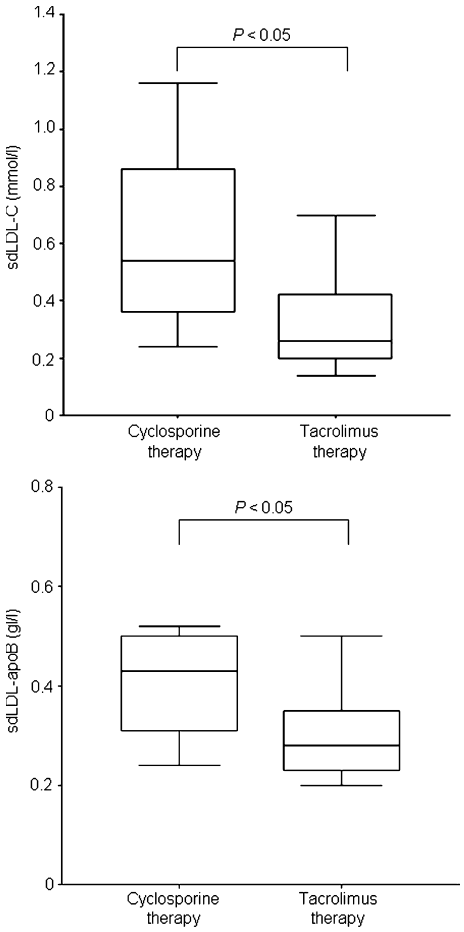

Figure 3 Concentrations of sdLDL-C and sdLDL-apoB in renal transplant recipients according to immunosuppressive therapy. Lower and upper lines indicate the 10th and 90th percentiles. The box indicates the 25th and 75th percentiles, while the line in the box indicates the median value. sdLDL-C, small, dense low-density lipoprotein-cholesterol; sdLDL-apoB, small, dense low-density lipoprotein-apolipoprotein B.

discrepancies in lipoprotein profiles of patients and controls were not clearly evident prior to the inclusion of HDL and LDL particle characteristics in the analysis. When taking these findings into account, it appears that traditional lipids and apolipoproteins measurements could not be sufficient for accurate and timely estimation of atherogenic dyslipidemia for this category of patients. The usefulness of advanced lipid testing could be particularly true for sdLDL-C, sdLDL-apoB, sdLDL-C (%), and sdLDL-apoB (%) measurement, which is a rather simple and nonexpensive method [15].

Previous studies have underlined the deleterious effect of a prolonged pretransplantation period on patients' survival [20,21]. Our current results could provide additional confirmation of these findings, implying a role of pretransplantation disease progress in adverse perturbations of HDL particle distributions. Namely, the proportion of large HDL 2b subclasses negatively correlated, while the proportion of small-sized HDL 3a particles positively correlated with the duration of prior chronic kidney disease (Table 3). Although HDL and its subclasses are generally regarded as cardioprotective, numerous studies [22,23] revealed a major rearrangement of HDL particle distribution in favor of smaller HDL subclasses in subjects with CVD. In attempting to explain such findings, it has been demonstrated that smaller HDL subclasses, even if they are essentially protective, can be highly vulnerable to possible detrimental effects of dyslipidemia, enhanced oxidative stress and inflammation [24]. Moreover, it has been suggested that such adverse conditions in the vascular environment could provoke certain impairments in the structure of small HDL particles, which in turn might decrease their anti-atherogenic capacity or even induce pro-atherogenic properties [5]. It has been shown that the uremic milieu affects HDL's ability to protect LDL from oxidation [25], its capacity for reverse cholesterol transport [26] and anti-inflammatory properties [27]. On the other hand, we found that renal transplant recipients had higher TG levels (Table 2). In addition, HDL size strongly inversely correlated with TG concentration, thus confirming a central role of hypertriglyceridemia in the formation of small-sized HDL particles. Our finding of increased CETP activity (Table 2) could provide additional insight into observed changes in HDL subclass distribution of the patients. Namely, in the case of elevated TG concentration, enhanced CETP activity mediates generation of smaller, TG-enriched HDL particles [24]. Based on the above-mentioned findings, we could speculate that previous uremia and later dyslipidemia, commonly seen in renal transplant recipients, together contribute to adverse modifications in the HDL particle profile, thereby further increasing CVD risk.

Preceding observations are further strengthened by strong positive association between small-sized HDL 3b particles and cIMT (Table 4). In previous studies regarding cIMT in pediatric renal transplant recipients [28,29], traditional lipid measurements were not recognized as independent predictors of increased cIMT, in contrast to similar studies in adults [30]. The authors [28,29] explained such inconsistency with the fact that the investigated patients were younger, with shorter duration of renal disease and consequently, with shorter exposure to hyperlipidemia. Similarly to Mitsefnes *et al.* [28], we found that systolic blood pressure was independently associated with cIMT. Further inclusion of measured LDL and HDL characteristics in the analysis revealed the proportion of small HDL 3b particles as another independent covariate. Obtained results suggest a role of lipid abnormalities in the development of arterial wall thickness in pediatric renal transplant

recipients, although such association might not be evident with regard to traditional lipid parameters.

Among CVD risk factors specific for transplantation, immunosuppression plays an important role. For this reason, we investigated the influence of different immunosuppressant drugs on HDL and LDL particle characteristics. A shift toward smaller, more atherogenic HDL and LDL particles was observed in patients who were treated with cyclosporine when compared with subjects treated with tacrolimus. However, statistical significance was reached only in the case of sdLDL-C and sdLDL-apoB (Fig. 3). It has been already shown that the conversion from cyclosporine to tacrolimus results in reduced serum LDL-C levels [10]. In addition, in the study by Apanay *et al.* [31], patients treated with higher cyclosporine levels showed significantly higher LDL oxidation susceptibility when compared with controls. In contrast, Cofan *et al.* [32] noted that tacrolimus therapy resulted in a LDL oxidation profile similar to that in healthy controls. Taking all together, we could conclude that conversion from cyclosporine to tacrolimus might have beneficial effects on both LDL particles distribution and susceptibility to oxidation, thereby reducing LDL's atherogenic potential. In contrast, we did not find significant differences in HDL size and subclasses profile between patients who received cyclosporine and those treated with tacrolimus. In a recent study, Seymen *et al.* [33] showed that switching from cyclosporine to tacrolimus may improve dyslipidemia by decreasing all serum lipid and lipoprotein concentrations. However, in the same study [33] the authors reported that tacrolimus administration had no effect on serum HDL 2/HDL 3 levels, which is in line with our current results.

There are some limitations to this study. First, cIMT measurement was not performed in the control group. Ideally, this study would have been better controlled by measuring cIMT in healthy children. However, this is not a common screening procedure at annual medical checkups. The main objective of this study was to evaluate LDL and HDL particle characteristics in pediatric renal transplant recipients, which may account for increased cIMT as a part of their presumed higher CVD risk. The reason for including the control group was to show that even with a small group of healthy children, LDL and HDL particle distributions are worse in pediatric renal transplant recipients. Another limitation is that, because of the cross-sectional design of the study, we could not prove a causal relationship between smaller HDL subclasses and higher cIMT values. However, this study may offer some basic observations that could form the basis for future in-depth studies.

In conclusion, our results demonstrated altered HDL and LDL particles characteristics, with a shift toward smaller, more atherogenic subfractions in pediatric renal transplant recipients. In addition, according to our data, alterations in the relative proportion of small-sized HDL 3b particles might influence cIMT. Finally, the results presented herein suggest the potential benefit of advanced lipid testing for monitoring risk of lipid abnormalities and planning therapy with the aim to improve patient's survival and quality of life.

Authorship

AZ and JV: performed analysis of lipoprotein subclasses and wrote the manuscript. VSK, ZJI and APA: conceived and designed the study and critically revised the manuscript. MK: undertook data collection and critically revised the manuscript. DV: acquired the data. SS: statistically analyzed data and critically revised the manuscript. All authors reviewed and approved the manuscript.

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