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Relationships between serum lipid, lipoprotein, triglyceride-rich lipoprotein, and high-density lipoprotein particle concentrations in post-renal transplant patients*

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Abstract: Objective: Disturbances in lipid and lipoprotein profiles in patients after kidney transplantation (Tx) are still not understood. Methods: Serum levels of lipids, lipoprotein, triglyceride-rich lipoproteins (TRLs), and high-density lipoprotein (HDL) particles were determined, lipid and lipoprotein ratios were calculated, and their relationships in Tx patients with hypertriglyceridemia (HTG) and lower apolipoprotein AI (apoAI) concentration were examined. Serum lipid and lipoprotein levels were measured in 109 Tx patients and 89 healthy subjects. HDL particle levels were determined by enzyme-linked immunosorbent assay (ELISA). Results: Tx patients had disturbed concentration, composition, and metabolism of TRLs and HDL particles. Multivariance analysis showed significant and positive correlation between HDL cholesterol/apoAI (HDL-C/apoAI) and HDL-C/HDL ratios, which indicates that both ratios could sensitively reflect changes in the HDL subclasses and their distribution into smaller size particles. In Tx patients, the decreased HDL-C/apoAI ratio indicates that, along with the decreased apoAI concentration, the HDL-C level is decreased. However, a low HDL-C/HDL ratio indicates that HDL particles in Tx patients transport lesser content of HDL-C but more triglyceride (TG) (high TG/HDL ratio), and thus are hypercatabolized and removed; therefore, concentration of HDL particles in serum was decreased. Conclusion: The decrease of HDL-C/apoAI ratio seems to be a good marker of HDL subclass distribution into smaller size particles.

Key words: Lipid, Lipoprotein, High-density lipoprotein particle, Triglyceride-rich lipoprotein, Renal transplantation
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1 Introduction

Post-renal transplant (Tx) patients exhibit a high cardiovascular morbidity and mortality due to accumulation of cardiovascular risk factors such as hypertension, hyperlipidemia, and post-transplantation diabetes mellitus. Genetic predisposition to hyperlipidemia, obesity and metabolic consequence of immunosuppressive, and anti-hypertension medications are factors of dyslipidemia in this kind of patients (Kasiske *et al*., 2000; Fellström, 2001; Kimak *et al*., 2006a; Shivaswamy *et al*., 2008). Apolipoprotein CIII (apoCIII), an important regulator of lipoprotein metabolism, is strongly associated with hypertriglyceridemia and the progression of cardiovascular disease (CVD). ApoCIII impairs lipolysis of triglyceride-rich lipoprotein (TRL) by inhibiting lipoprotein lipase (LPL) and the hepatic uptake of TRLs by remnant receptors. In the circulation, apoCIII is associated with TRL and high-density lipoprotein (HDL), and freely exchanges among these lipoprotein particle systems. Experimental evidence shows that apoCIII may also have a direct role in atherosclerosis (Ooi *et al*., 2008). Unfortunately, there is little

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information about concentrations and metabolism of TRL and apoCIII in HDL, and HDL particle concentration in patients.

In this study, the serum levels of lipids, TRLs (apoB:CIII, apoB:E), apoB as non-HDL lipoproteins as well as apoAI, apoCIIInonB and apoEnonB in the HDL fraction, and HDL particles were determined. Lipid and lipoprotein ratios were calculated, and their relationships in Tx patients with hypertriglyceridemia and lower apoAI concentrations were examined.

2 Materials and methods

2.1 Participants

A total of 109 renal transplant recipients (male and female) aged 21–60 years and 89 apparently normolipidemic healthy individuals as control were recruited in the current study. The study was conducted in accordance with the guidelines of the Ethics Committee, Medical University of Lublin, Poland. The studied patients were without active inflammatory disease, liver disease, malignancy, or diabetes mellitus, and they were not smokers. Sixty-nine patients had hypertension. The causes of renal insufficiency in the post-renal transplant (Tx) patients with dyslipidemia were: 58 glomerulonephritis, 6 interstitial nephritis, 10 polycystic disease, 3 hypertensive nephrosclerosis, 7 congenital defect, and 25 unknown. The Tx patients received cyclosporine A (CsA)+ prednisone (n=76), tacrolimus+prednisone (n=33), and atorvastatin or simvastatin (n=58). They received low dose of statins. Fifty-one patients were without anti-lipid drug treatment because 33 had minimal lipids disturbances, 17 with normolipidemia did not require anti-lipid lowering therapy, and 2 received medicines irregularly. Tx patients were divided into two groups: Tx patients with (n=58) and without (n=51) statin therapy. All Tx patients were also divided into another two groups: patients with apoAI<1500 mg/L and apoAI>1500 mg/L, using apoAI concentration of 1500 mg/L as a cut-point (Contois *et al*., 1996; National Kidney Foundation, 2002; Tseng, 2006; Kasiske *et al*., 2004). The same Tx patients were further divided into two groups: patients with triglyceride (TG)>1500 mg/L and TG<1500 mg/L, using TG concentration of 1500 mg/L as a cut-point. Recommendation for diabetic patients with renal dyslipidemia suggests that plasma TGs should be reduced to a level of 1500 mg/L (<1.7 mmol/L) (Contois *et al*., 1996; National Kidney Foundation, 2002; Kasiske *et al*., 2004; Tseng, 2006), and thus the TG cut-point was decided to be 1500 mg/L. Hypertensive patients were treated with anti-hypertensive medications of either calcium channel blockers or angiotensin converting enzyme antagonists, and AT1- and α-blockers, but not diuretics. The patients who received β-blockers and statins remained in all studied groups.

2.2 Detection of lipids and lipoproteins

Lipids, lipoproteins, and routine laboratory parameters were obtained in serum after a 14-h overnight fasting. Blood was taken from veins into commercial tubes. Serum was immediately separated and stored in aliquots at −80 °C until use. Routine laboratory parameters (the level of urea, uric acid, creatinine, total protein, and albumin), lipids and lipoproteins (apoA, apoB) were determined on a Hitachi 902 analyzer, and hemoglobin using an ADVIA analyser (Bayer, HealthCare, Germany), as previously described (Kimak *et al*., 2006a; 2006b; 2007). Low-density lipoprotein-cholesterol (LDL-C) was calculated according to the Friedewald formula (Friedewald *et al*., 1972). Non-HDL-C was calculated as total cholesterol (TC) minus HDL-C. Lipoproteins (total apoCIII, apoCIIInonB, total apoE and apoEnonB) were measured by electroimmunodiffusion according to Laurell method using a commercial kit (Sebia, USA) as previously described (Kimak *et al*., 2006a; 2006b; 2007).

2.3 Detection of HDL particle concentration

HDL concentration was measured using the enzyme-linked immunosorbent assay (ELISA) method in our laboratory. The method was based on specific direct immunological reaction between purified chicken anti-human HDL antibodies and human HDL (GenWay Biotech Inc., USA). The antibodies react specifically to human HDL, but not other human serum proteins. A 96-well microtiter plate was coated with captured antibody diluted in coating buffer (0.05 mol/L carbonate-bicarbonate, pH 9.6) and incubated at room temperature for 60 min. After washing with wash solution (50 mmol/L Tris, 0.14 mol/L NaCl-Tween 20 buffer, pH 8.0), the plate was blocked with

1% (w/v) milk in phosphate buffered saline (PBS), incubated at room temperature for 60 min, and well washed three times with wash solution. Serum samples and standards (pure human HDL antigen) were diluted (1.2–5000 ng/ml) before they were added to the wells. After 1 h incubation at room temperature, the samples and standards were removed and washed five times with wash solution, and the conjugated antibody (purified chicken anti-human HDL-horse radish peroxidase (HRP) conjugate) was added and incubated at room temperature for 60 min. After washing, Tetra-methyl-benzidine (TMB) was added as a substrate. The reaction was stopped by adding 2 mol/L H_2SO_4 and measured at 450 nm. The readings were for duplicate standards, samples and controls, and average values were calculated. A standard curve was made from the standard data, from which the concentration of HDL particles was read for each sample and control.

2.4 Statistical analysis

Statistical analysis was performed using the STATISTICA 8.0 program (StatSoft, Krakow, Poland). The values are expressed as median (min–max). The differences among the groups of subjects were evaluated by Kruskal-Wallis test. Correlation between variables was calculated by non-parametric Spearman rank coefficient test. Multivariance regression analysis was used to investigate the relationships between the concentration of HDL particles and HDL-C/apoAI ratio and between the concentration of lipoproteins and lipid and lipoprotein ratios. In the model of multiple forward stepwise regression analysis for variable, HDL particle concentration and HDL-C/apoAI ratio were selected as the independent variables, and for each dependent variable, parameters were calculated according to the equation: $y=\beta_0x_1+\beta_0x_2+\ldots+\beta_nx_n$. The relationship between the independent and dependent variables is expressed by the coefficient of multiple regression (β), which gives information about the relationship between the independent HDL particle concentration and HDL-C/apoAI ratio variables and the dependent variables. The statistical significance of all variables was established at $P<0.05$.

3 Results

3.1 Basic information of subjects

Table 1 presents the results of the clinical and laboratory parameters in all Tx patients. Table 2 shows that Tx patients treated with statins had worse clinical and laboratory parameters than Tx patients without these medicaments.

3.2 Concentrations of serum lipids, lipoproteins and HDL particles and lipid and lipoprotein ratios

Tx patients receiving statins had worse TG, apoCIII, and apoB:CIII concentrations as well as worse lipid and lipoprotein ratios when compared with Tx patients without statin therapy and controls (Table 3). The Tx patients with apoAI<1500 mg/L and TG>1500 mg/L (Tables 4 and 5) had significantly

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

Parameter	Tx patients (n=109)	Reference group (n=89)
Age (year)	47 (18–68)	46 (22–69)
Sex	59 M, 50 F	44 M, 45 F
BMI (kg/m^2)	24.7 (16.8–44.1)	21.5 (18.5–25.3)
Time after transplant (month)	29.7 (15.2–185.2)	–
eGFR (ml/(min·1.73 m^2))	67.3 (23.3–123.4)	–
Number of patients		
Prednisone	109	–
Prograf	33	–
Cyclosporine A	76	–
Statin	57	–
Urea (mmol/L)	8.15 (3.54–24.9)**	4.40 (2.54–6.42)
Creatinine (μmol/L)	130 (71–486)**	75 (62–102)
Total protein (g/L)	71.3 (57.0–86.0)	73.0 (71.2–78.0)
Albumin (g/L)	42.0 (29.3–48.9)	45.0 (42.1–48.4)
Hemoglobin (mmol/L)	8.68 (5.15–10.76)	9.00 (8.38–10.12)

BMI: body mass index; eGFR: estimated glomerular filtration rate; M: male, F: female. Values are expressed as median (min–max); ** $P<0.01$ vs. the reference group

Table 2 Clinical and routine laboratory parameters in post-renal transplant (Tx) patients with and without statin therapy and the reference group

Parameter	Tx patients with statins (n=58)	Tx patients without statins (n=51)	Reference group (n=89)
Age (year)	51 (30–68)	40 (18–58)	46 (22–69)
Sex	31 M, 27 F	28 M, 23 F	44 M, 45 F
BMI (kg/m^2)	24.5 (18.2–36.1)	23.3 (17.6–26.2)	21.5 (18.5–25.3)
Time after transplant (month)	30.7 (25.1–123.4)	28.0 (19.1–175.3)	–
eGFR (ml/(min·1.73 m^2))	62 (23–123)	68 (38−94)	–
Number of patients			
Prednisone	57	51	–
Prograf	14	14	–
Cyclosporine A	41	35	–
Statin	57	–	–
Urea (mmol/L)	8.96 (3.55−24.90)**	7.64 (3.70−24.90)**	4.40 (2.54−6.42)
Creatinine (μmol/L)	142 (90−486)**	126 (81−320)**	75 (62−102)
Total protein (g/L)	71.0 (60.1−86.0)	72.1 (63.0−83.2)	73.0 (71.12−78.0)
Albumin (g/L)	42.0 (35.0−47.0)	42.0 (35.0−48.1)	45.0 (42.1−48.4)
Hemoglobin (mmol/L)	8.63 (6.39−10.0)	8.87 (6.52−10.36)	9.00 (8.38−10.12)

BMI: body mass index; eGFR: estimated glomerular filtration rate; M: male, F: female. Values are expressed as median (min–max); ** P<0.01 vs. the reference group

Table 3 Concentrations of lipids and lipoproteins and lipid and lipoprotein ratios in post-renal transplant (Tx) patients with and without statin therapy and the reference group

Parameter	Tx patients with statins (n=58)	Tx patients without statins (n=51)	Reference group (n=89)
TG (mmol/L)	2.15 (0.70–3.15)***†	1.88 (0.50–3.15)***	0.93 (0.40–1.70)
TC (mmol/L)	5.18 (3.57–7.22)	4.99 (3.70–6.73)	4.58 (3.14–5.31)
LDL-C (mmol/L)	3.08 (1.71–5.13)	2.98 (1.55–4.74)	2.69 (1.32–4.22)
HDL-C (mmol/L)	0.98 (0.65–2.05)***	0.98 (0.65–1.99)***	1.48 (1.24–2.12)
Non-HDL-C (mmol/L)	4.07 (2.75–6.48)***	3.89 (2.72–5.47)**	3.13 (1.81–3.50)
HDL (mg/L)	21.8 (10.5–36.5)	22.5 (11.4–38.0)	23.6 (18.5–38.1)
ApoAI (mg/L)	1540 (300–1990)	1550 (1070–1990)	1610 (1380–1860)
ApoB (mg/L)	910 (660–1380)*	860 (570–1280)	740 (600–1180)
ApoCIII (mg/L)	51 (22–85)***†	44 (23–90)**	27 (22–33)
ApoCIIInonB (mg/L)	30 (16–66)**	30 (15–57)**	19 (15–25)
ApoCIII:B (mg/L)	20 (6–50)***†	14 (8–40)**	7 (3–10)
ApoE (mg/L)	57 (30–110)	56 (30–138)	49 (35–83)
ApoEnonB (mg/L)	45 (25–84)	48 (25–105)	39 (27–49)
ApoE:B (mg/L)	14 (3–45)	13 (4–55)	11 (5–24)
TC/HDL-C	5.26 (2.89–9.65)***	5.09 (3.04–8.14)***	2.96 (1.20–4.41)
LDL-C/HDL-C	2.90 (1.49–6.87)***	3.08 (1.5–5.48)***	1.81 (0.8–3.46)
TG/HDL-C	5.42 (1.21–12.35)***	4.37 (0.85–8.65)***	1.21 (0.52–2.61)
ApoAI/apoB	1.69 (1.00–2.72)**	1.83 (1.10–2.95)*	2.31 (1.56–2.57)
ApoAI/apoCIII	30.2 (15.0–67.0)***	35.2 (16.0–75.0)***	58.6 (50.4–67.5)
HDL-C/HDL	17.1 (9.3–42.6)*	17.0 (10.2–50.4)*	24.1 (22.3–27.6)
HDL-C/apoAI	0.25 (0.17–0.39)***	0.25 (0.18–0.39)***	0.35 (0.29–0.37)
TG/HDL	87 (35–210)**	74 (27–240)**	36 (28–51)

TG: triglyceride; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein. Values are expressed as median (min–max); * P<0.05, ** P<0.01, *** P<0.001 vs. the reference group; † P<0.05, †† P<0.01, ††† P<0.001 vs. the Tx patients with statins

Table 4 Concentrations of lipids and lipoproteins and lipid and lipoprotein ratios in post-renal transplant (Tx) patients with apoAI<1500 mg/L and apoAI>1500 mg/L and the reference group

Parameter	Tx patients with apoAI<1500 mg/L (n=58)	Tx patients with apoAI>1500 mg/L (n=51)	Reference group (n=89)
TG (mmol/L)	1.98 (1.00–3.95)***	1.93 (0.51–3.12)***	0.93 (0.40–1.70)
TC (mmol/L)	5.00 (3.70–7.22)	5.21 (3.57–7.22)	4.58 (3.14–5.31)
LDL-C (mmol/L)	3.05 (1.55–5.12)	2.98 (1.73–4.74)	2.69 (1.32–4.22)
HDL-C (mmol/L)	0.85 (0.65–1.17)***†††	1.21 (0.80–2.07)***	1.48 (1.24–2.12)
Non-HDL-C (mmol/L)	4.07 (2.77–6.48)***	4.02 (2.72–5.93)***	3.13 (1.81–3.50)
HDL (mg/L)	21.4 (10.5–36.1)*	22.5 (11.4–38.1)	23.6 (18.5–38.1)
ApoAI (mg/L)	1420 (1070–1500)***†††	1720 (1530–1990)	1610 (1380–1860)
ApoB (mg/L)	910 (600–1380)*	870 (570–1320)	740 (600–1180)
ApoCIII (mg/L)	47 (22–90)***	47 (23–85)***	27 (22–33)
ApoCIIInonB (mg/L)	28 (15–66)**	30 (20–65)**	19 (15–25)
ApoCIII:B (mg/L)	19 (6–50)***†	17 (8–34)**	7 (3–10)
ApoE (mg/L)	55 (30–110)	58 (30–138)	49 (35–83)
ApoEnonB (mg/L)	41 (25–84)	45 (25–105)	39 (27–49)
ApoE:B (mg/L)	13 (30–49)	13 (4–55)	11 (5–24)
TC/HDL-C	5.62 (3.68–9.65)***†††	4.23 (2.82–6.37)***	2.96 (1.20–4.41)
LDL-C/HDL-C	3.55 (1.50–6.86)***†††	2.41 (1.49–4.38)***	1.81 (0.80–3.46)
TG/HDL-C	5.31 (2.83–12.35)***†††	3.81 (0.84–7.21)***	1.21 (0.52–2.61)
ApoAI/apoB	1.55 (1.00–2.68)***††	1.93 (1.19–2.95)	2.31 (1.56–2.57)
ApoAI/apoCIII	29.89 (15.71–67.73)***††	36.51 (19.81–70.50)***	58.57 (50.35–67.53)
HDL-C/HDL	15.11 (9.30–32.18)**††	19.93 (11.70–50.43)*	24.05 (22.31–27.63)
HDL-C/apoAI	0.23 (0.16–0.29)***††	0.28 (0.20–0.41)***	0.35 (0.29–0.37)
TG/HDL	82 (34–219)***	76 (27–164)**	36 (28–51)

TG: triglyceride; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein. Values are expressed as median (min–max); * P<0.05, ** P<0.01, *** P<0.001 vs. the reference group; † P<0.05, †† P<0.01, ††† P<0.001 vs. the Tx patients with apoAI<1500 mg/L

Table 5 Concentrations of lipids and lipoproteins and lipid and lipoprotein ratios in post-renal transplant (Tx) patients with TG>1500 mg/L and TG<1500 mg/L and the reference group

	Tx patients with TG>1500 mg/L (n=71)	Tx patients with TG<1500 mg/L (n=38)	Reference group (n=89)
TG (mmol/L)	2.26 (1.77–3.96)***†††	1.46 (0.51–1.70)***	0.93 (0.40–1.70)
TC (mmol/L)	5.20 (3.57–7.23)*†	4.58 (3.70–6.73)	4.58 (3.14–5.31)
LDL-C (mmol/L)	3.13 (1.55–5.13)*†	2.82 (2.20–4.74)	2.69 (1.32–4.22)
HDL-C (mmol/L)	1.01 (0.64–2.05)***	1.08 (0.67–1.99)***	1.48 (1.24–2.12)
Non-HDL-C (mmol/L)	4.20 (2.75–6.48)***†††	3.55 (2.72–5.31)**	3.13 (1.81–3.50)
HDL (mg/L)	21.1 (10.5–31.1)*	22.5 (10.5–35.6)	23.6 (18.5–38.1)
ApoAI (mg/L)	1540 (1070–1990)	1540 (1220–1990)	1610 (1380–1860)
ApoB (mg/L)	920 (570–1380)*†	801 (600–1280)	740 (600–1180)
ApoCIII (mg/L)	50 (23–90)***	41 (22–80)**	27 (22–33)
ApoCIIInonB (mg/L)	31 (17–65)**	28 (15–66)**	19 (15–25)
ApoCIII:B (mg/L)	19 (6–50)***†	13 (8–30)**	7 (3–10)
ApoE (mg/L)	57 (37–110)	51 (30–138)	49 (35–83)
ApoEnonB (mg/L)	45 (25–84)	37 (25–105)	39 (27–49)
ApoE:B (mg/L)	14 (3–55)	11 (4–38)	11 (5–24)
TC/HDL-C	5.23 (3.07–9.65)***††	4.64 (2.82–6.87)***	2.96 (1.20–4.41)
LDL-C/HDL-C	3.22 (1.51–6.86)***†	2.79 (1.49–4.80)***	1.81 (0.80–3.46)
TG/HDL-C	5.11 (2.67–12.35)***†††	3.20 (0.84–7.86)***	1.21 (0.52–2.61)
ApoAI/apoB	1.67 (1.00–2.68)**†	1.82 (1.24–2.95)*	2.31 (1.56–2.57)
ApoAI/apoCIII	30.90 (18.10–48.02)***†	37.50 (18.10–67.05)***	58.57 (50.35–67.53)
HDL-C/HDL	18.3 (9.3–42.0)*	18.7 (10.2–50.4)*	24.1 (22.3–27.6)
HDL-C/apoAI	0.25 (0.17–0.41)***	0.27 (0.19–0.38)***	0.35 (0.29–0.37)
TG/HDL	94 (49–249)***†††	57 (26–131)*	36 (28–51)

TG: triglyceride; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; Apo: apolipoprotein. Values are expressed as median (min–max); * P<0.05, ** P<0.01, *** P<0.001 vs. the reference group; † P<0.05, †† P<0.01, ††† P<0.001 vs. the Tx patients with TG>1500 mg/L

increased concentrations of TG, LDL-C, non-HDL-C, apoB, apoCIII, apoCIIInonB, apoB:CIII, and lipid (TC/HDL-C, LDL-C/HDL-C, TG/HDL-C) and lipoprotein (TG/HDL) ratios, and presented decreased levels of HDL-C, apoAI and HDL particles, and lipoprotein ratios (apoAI/apoB, HDL-C/apoAI, apoAI/apoCIII, HDL-C/HDL) in comparison with the reference group. However, TC, LDL-C, apoB, apoE, apoEnonB, and apoB:E were moderately increased. Lipid and lipoprotein profile parameters and ratios were significantly more beneficial in the Tx patients with apoAI>1500 mg/L and TG<1500 mg/L than in Tx patients with apoAI<1500 mg/L and TG>1500 mg/L, but worse than those in controls.

3.3 Relationships between concentrations of lipids and lipoproteins and lipid and lipoprotein ratios

Correlation between variables was calculated by non-parametric Spearman rank coefficient test. The concentration of apoAI was significantly positively correlated with HDL-C (R=0.872, P<0.001), HDL particle levels (R=0.336, P<0.05), apoAI/apoB (R=0.486, P<0.001), apoAI/apoCIII (R=0.356, P<0.004), HDL-C/apoAI (R=0.668, P<0.001), and HDL-C/HDL (R=0.359, P<0.003) ratios, but significantly negatively correlated with TC/HDL-C (R=−0.755, P<0.001), LDL-C/HDL-C (R=−0.678, P<0.001), TG/HDL-C (R=−0.747, P<0.001), and TG/HDL (R=−0.275, P<0.05) ratios. HDL particle concentration was significantly positively correlated with apoAI levels but significantly negatively with TG (R=−0.321, P<0.01), TG/HDL-C (R=−0.334, P<0.01), TG/HDL (R=−0.871, P<0.001), and HDL-C/HDL (R=−0.762, P<0.001) ratios. Multivariance regression demonstrated that apoAI was associated independently and positively with HDL particle concentration and it was the most potent predictor for alterations of HDL concentration. However, HDL-C/HDL and TG/HDL ratios showed a negative correlation with HDL particle concentration, but HDL-C/apoAI ratio showed a significant positive correlation with HDL-C/HDL and significant negative correlation with TG/HDL ratio (Tables 6 and 7).

Table 6 Multivariance regression between concentration of HDL particles and lipoprotein levels and lipoprotein ratios in Tx patients

	HDL particle correlation	
	β	P
ApoAI (mg/dl)	0.355	0.007
HDL-C/HDL	–0.620	0.001
TG/HDL	–0.340	0.0003

Table 7 Multivariance regression between HDL-C/apoAI ratio and lipoprotein ratios in Tx patients

	HDL-C/apoAI ratio correlation	
	β	P
HDL-C/HDL	0.873	0.0003
TG/HDL	–0.60	0.0001

4 Discussion

The cardiovascular risk is improved by kidney transplant (KTX) but remains the leading cause of mortality after KTX and is 50-fold higher in patients after kidney transplantation than in general population. A number of studies have shown that hyperlipidemia may contribute to a high incidence of allograft dysfunction and subsequent rejection (Kasiske *et al*., 2000; Shivaswamy *et al*., 2008). After renal transplantation, various types of metabolic dysfunctions are associated with reverse chronic renal failure, but lipid abnormalities appear to progress in a large fraction of patients. The typical pattern includes marked hypercholesterolemia and hypertriglyceridemia as the consequence of immunosuppressive therapy (Kasiske *et al*., 2000; Wissing *et al*., 2000).

Our studies showed that Tx patients had dyslipoproteinemia and dyslipidemia that were characterized by increased concentration of TRLs and decreased concentration of HDL particles as well as disturbed lipid and lipoprotein ratios. Furthermore, we observed that, along with TRL (apoB:CIII) accumulation, ApoA-I level and apoAI/apoCIII, HDL-C/apoAI, HDL-C/HDL and apoAI/apoB ratios were decreased. Moreover, TG concentration and TG/HDL-C and TG/HDL ratios indicated that HDL particles contained a higher content of TG in the studied patients than in the controls, and in turn, the composition and concentration of TRLs were disturbed (Kimak *et al*., 2006b; 2007). Also apoAI/apoCIII, apoAI/apoB, HDL-C/apoAI and HDL-C/HDL ratios suggested that HDL particles had lower contents of apoAI and HDL-C. These disturbances

exerted an influence on metabolism and concentration of HDL particles. We demonstrated that Tx patients with dyslipidemia, particularly with hypertriglyceridemia along with decreased apoAI concentration (<1500 mg/L), had decreased concentrations of HDL-C and HDL particles (positive correlation between concentrations of apoAI and HDL-C and HDL) and decreased ratios of HDL-C/apoAI, HDL-C/HDL, apoAI/apoCIII and apoAI/apoB. However, the ratios of TC/HDL-C, LDL-C/HDL-C, TG/HDL-C and TG/HDL were increased and negatively correlated with apoAI concentration, which suggested disordered concentration and composition of HDL particles that are poor in cholesterol esters but enriched in TGs (Gou *et al*., 2005; Yang *et al*., 2005; Jia *et al*., 2006). As a matter of fact, although Tx patients with apoAI<1500 mg/L and TG>1500 mg/L had lower HDL particle and higher apoB:CIII concentrations, in Tx patients with apoAI>1500 mg/L and TG<1500 mg/L, apoB:CIII concentration was moderately increased and HDL particle concentration was moderately decreased compared with controls. However, all Tx patients had disturbed lipid and lipoprotein metabolisms of TRLs and HDL particles. Our clinical observations and laboratory results are consistent with others (Gou *et al*., 2005; Yang *et al*., 2005; Jia *et al*., 2006).

We conducted multivariate regression analysis to assess the independent association between HDL particle and lipoprotein concentrations and ratios. HDL particle concentration and particularly ratios of HDL-C/apoAI, HDL-C/HDL and TG/HDL could reflect sensitive changes in the HDL subclasses. Our results demonstrated that variable concentration of HDL particles depends on the variability of apoAI concentration (positive correlation) and variability of HDL-C/HDL and TG/HDL ratios (negative correlation). Along with the increase in TG content in HDL (TG/HDL ratio), HDL concentration as well as particle size was decreased. Hence, HDL particles were poorer in apoAI, which may suggest that their maturation might be affected by the blocked HDL particles (decreased HDL-C/HDL ratio). Thus the particles are faster catabolized and removed from the circulation by which reverse cholesterol transport (RCT) is weaker. The apoAI concentration and especially ratios of HDL-C/HDL and HDL-C/apoAI (positive correlation between HDL-C/apoAI and HDL-C/HDL ratios) could distinctly reflect the alteration of HDL subclass distribution. Simultaneous increases of TG/HDL and TG/HDL-C ratios and decreases of HDL-C/apoAI and HDL-C/HDL ratios could be good markers of HDL size disorder into smaller size particles.

Disturbance in TRL metabolism is also known to exert impact on HDL-apoAI metabolism (Rashid *et al*., 2002; Jia *et al*., 2007; Chan *et al*., 2008). According to Chan *et al*. (2008), TG-rich HDL, generated by increased neutral lipid exchange with TG-rich very low density lipoprotein (VLDL), is a preferred substrate for hepatic lipase, which accelerates the catabolism of these thermodynamically unstable HDL particles. The functional role of apoCIII in inhibiting the hydrolysis of TGs indicates that accumulation of apoCIII in plasma will favor the formation of unstable TG-rich HDL particles, thereby increasing the catabolism of HDL-apoAI. Therefore, increased concentration of TRLs in Tx patients is an independent determinant of hypercatabolism of HDL-apoAI and by implication low plasma HDL-C, a risk factor for coronary heart disease and renal recipient rejection (Kimak *et al*., 2006b; Chan *et al*., 2008). Plasma concentrations of HDL-C and apoAI are inversely correlated with plasma TG concentration. ApoAI can activate lecithin cholesterol acyltransferase (LCAT), and LCAT may catalyze unesterified cholesterol to cholesterol ester promoting conversion of preβ1-HDL and HDL3 to HDL2. Hence, the reduction of apoAI levels results in increased percentage of small-size HDL particles and decreased percentage of large-size HDL particles (Rubin *et al*., 1991; Brinton *et al*., 1994; Jia *et al*., 2007). Tian *et al*. (2008) indicated the effect of apoB100 combined with apoAI levels on changes in HDL subclass distribution. Therefore, apoAI levels might reflect the number of HDL particles. With the reduction of apoAI levels, molecules of apoAI distributed to the each subclass decreased, resulting in a decrease in the total number of HDL particles. Recently, Tian *et al*. (2009) indicated that the particle size of HDL shifted towards smaller sizes with increase of plasma apoCIII levels, and the shift was more remarkable when the elevation of apoCIII and apoCII was simultaneous. Besides, the higher apoAI concentrations could modify the effect of apoCIII on HDL subclass distribution profile. Large-size HDL_{2b} particles decreased greatly in hypertriglyceridemic

subjects who were characterized by elevated apoCIII and apoCII accompanied with significantly lower apoAI, which, in turn, blocked the maturation of HDL (Tian *et al*., 2009).

HDL has different antiatherogenic potential and functional properties. It was reported that both HDL size and HDL particle concentration were independently associated with other cardiovascular risk factors and the risk for coronary artery disease (Harchaoui *et al*., 2009). The researchers suggested that lipolysis of VLDL particles by lipoprotein lipase is an important source for formation of preβ1-HDL. Preβ1-HDL particle was generated during lipolysis of TRLs by lipoprotein lipase (LPL) (Miyazaki *et al*., 2009). The findings of TRLs and TRL remnants in atheromatous plaques provide critical evidence supporting their direct roles in atherogenesis (Ooi *et al*., 2008). Recently, it was shown that TRL lipolysis releases neutral and oxidized free fatty acids (FFAs) that induce endothelial cell inflammation. Therefore, the oxidative metabolism of FFA in endothelial cells can produce inflammatory responses. TRL lipolysis can also release mediators of oxidative stress that may influence endothelial cell function in vivo by stimulating intracellular reactive oxygen species (ROS) production (Wang *et al*., 2009). Moreover, it was suggested that CsA administration may decrease the antioxidant capacity of renal tissue (Ghaznavi *et al*., 2007). In another study, hypertriglyceridemia was found to be associated with a greater probability of doubling serum creatinine often recognized as a major contributor to renal allograft dysfunction (Kasiske *et al*., 2000; Wissing *et al*., 2000; Shivaswamy *et al*., 2008). Meanwhile, it is one of the most common metabolic disorders in kidney transplant recipients and also an independent risk factor for renal allograft nephropathy. It was also observed that insulin resistance level in recipients with hypertriglyceridemia and hypercholesterolemia was higher than that in recipients without these disorders (Sui *et al*., 2008).

5 Conclusion

Dyslipoproteinemia, which is primary to dyslipidemia in Tx patients, suggests disturbed concentration, composition, and metabolism of TRLs and HDL particles. The decrease of apoAI and HDL concentrations, particularly HDL-C/apoAI and HDL-C/HDL ratios, and the increase of TG/HDL-C and TG/HDL ratios could sensitively reflect changes in the HDL subclasses and their distribution to smaller size particles. It may also suggest atherosclerosis risk and graft rejection in Tx patients. We conclude that, except well known dyslipidemia markers like the decreased concentration of apoAI, the increased apoB level and low apoAI/apoB ratios also decrease HDL-C/apoAI ratio and appear to be a good marker for the distribution of HDL subclass into smaller size particles.

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