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 A1 (apoA1), 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...
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:AI, 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, AIInvB, apoB-containing AI (apoB:AI), apoCIII, apoCIINvB, 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+Azatioprine, CsA+CellCept; the patients were given CsA, and Prograf + Myfortic and Prograf+CellCept and CellCept+Myfortic. Tx patients without statin therapy received mainly Prograf (60% of Tx patients) in connection with Myfortic, CellCept and CsA+Myfortic, together with CsA+Azatioprine 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, apoAIInvB, 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 apoCIINvB were measured by electroimmunodiffusion, according to Laurell, using a commercial kit (Sebia, USA), TRLs (apoB:CIII and apoB:AI) 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)**

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

Values are expressed as median (min-max); * p<0.05 vs. the reference group.
The relationship between the dependent variables is expressed by the coefficient of multiple regression ($\beta$), 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 dyslipidaemia and dyslipoproteinaemia 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:CII 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 > 150 mg/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 apoCIIImonB ($\beta = 0.535$, $P = 0.003$) 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 apoAII/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 non-HDL-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 apoCIIImonB 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)

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

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

78
The results of the presented studies and those by other laboratories have already shown that TG-rich apoB-containing apoCII 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 particles.

The 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 hemodynamics 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 particle 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].
and (c) decreases the PON1 stimulation 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/apoCII 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.

REFERENCES

9. Hine D., Mackness B., Mackness M.: Coincubation of PON1, apoAI, and LCAT increases the time HDL is able to prevent LDL oxidation. IUBMB Life, 64, 157, 2012.