LIPOPROTEIN METABOLISM ABNORMALITIES IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY

ABNORMALNOSTI U METABOLIZMU LIPOPROTEINA KOD PACIJENATA SA HRONIČNOM BUBREŽNOM INSUFICIJENCIJOM

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Summary: Patients with chronic renal insufficiency (CRI) on hemodialysis develop lipoprotein abnormalities that may contribute to increased risk for atherosclerosis. The objective of this study was to assess the atherogenic risk of chronic renal insufficiency patients and dialysis treated patients (DTP) by measuring total cholesterol (TC), triglycerides (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and calculating the risk factor ratio: TC/HDL-C and LDL-C/HDL-C. The examined group consisted of 18 chronic renal insufficiency patients and 60 patients on hemodialysis. The results were compared to a control group of 85 voluntary blood donors. Serum lipid parameters were examined by standard methods. All lipid parameters in hemodialysis patients were statistically different as compared to the control group (p<0.05) while chronic renal insufficiency patients showed significant difference only in triglycerides and HDL-cholesterol. Hypertriglyceridemia was present in both examined groups of patients and HDL-cholesterol was lower within both groups. All calculated atherogenic ratios were higher for patients than the control group. Lipid parameters were compared between chronic renal insufficiency and hemodialysis patients, but statistically significant difference was obtained only for HDL-cholesterol (p<0.05). The increased values of triglycerides and lower HDL-cholesterol in chronic renal insufficiency patients contribute to high incidence of cardiovascular disease. Chronic renal insufficiency patients have impaired reverse cholesterol transport from peripheral cells to lipoproteins, decreased levels of HDL-cholesterol, hypertriglyceridemia prevalence of small, dense LDL and increased levels of potentially atherogenic remnant particles.

Keywords: chronic renal disease, hemodialysis, lipoprotein

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Kratak sadržaj: Kod pacijenata sa hroničnom bubrežnom insuficijencijom (CRI) na hemodijaliziraju se lipoproteinske abnormalnosti koje mogu doprineti povećanom riziku za aterosklerozu. Cilj ove studije bio je da se ispita aterogeni rizik kod pacijenata sa hroničnom bubrežnom insuficijencijom i pacijenata lećenih dijalizom (DTP) merenjem ukupnog holesterola (TC), triglicerida (TG), HDL- holesterola (HDL-C), LDL-holesterola (LDL-C) i izračunavanjem odnosa faktora rizika: TC/HDL-C, LDL-C/HDL-C. Grupu ispitanika činilo je 18 pacijenata sa hroničnom bubrežnom insuficijencijom i 60 pacijenata na hemodijalizi. Rezultati su upoređeni sa kontrolnom grupom od 85 dobrovoljnih davalaca krvi. Lipidni parametri u serumu ispitanika su mereni standardnim metodama. Svi lipidni parametri kod pacijenata lećenih hemodijalizom su statistički značajno različiti u odnosu na kontrolnu grupu (p<0.05), dok su pacijenti sa hroničnom bubrežnom insuficijencijom pokazali statistički značajne razlike samo u trigliceridima i HDL-cholesterolu. Hipertrigliceridemija bila je prisutna u obe ispitane grupe pacijenata a HDL-cholesterol bio je niži u okviru obe grupe. Svi izračunati aterogeni odnosi bili su viši za pacijente nego za kontrolnu grupu. Upoređeni su lipidni parametri kod pacijenata sa hroničnom bubrežnom insuficijencijom i pacijenata lećenih hemodijalizom, ali statistički značajna razlika je dobijena samo za HDL-cholesterol (p<0.05). Povišene vrednosti triglicerida i niži HDL-cholesterol kod pacijenata sa hroničnom bubrežnom insuficijencijom doprinose visokoj incidenciji kardiovaskularnih bolesti. Kod pacijenata sa hroničnom bubrežnom insuficijencijom oštećen je reverzni transport holesterola od perifernih komada do lipoproteina, snažni su nivoi HDL-cholesterola, prisutna je hipertrigliceridemija uz prevalencu malih gustih LDL i povišeni su nivoi potencijalno aterogenih ostataka (remnant čestica).

Ključne reči: hronično renalno oboljenje, hemodializa, lipoprotein
Introduction

Atherosclerosis and cardiovascular disturbance are common among patients with progressive renal insufficiency and in uremic patients receiving long term hemodialysis (1, 2). Cardiovascular disease is the most important cause of mortality in end-stage renal disease (3). Early reports suggested that atherosclerosis is accelerated during hemodialysis for end-stage renal disease. The mechanism for developing atherosclerosis in chronic renal insufficiency and hemodialysis is multi-factorial. However, plasma lipid disturbances have been identified as significant risk factors for cardiovascular disease in these patients. Previous studies have revealed that progressive renal failure is accompanied by abnormalities of the lipoprotein transport and that renal insufficiency is predominantly reflected in altered concentrations and composition of individual lipoproteins (4). The main lipid abnormality is an increase in plasma triglyceride and a decrease in HDL-cholesterol concentrations, with smaller change in the levels of cholesterol rich lipoproteins. The principle features of the impaired lipoprotein metabolism include the increase in very-low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) fractions, which are mainly due to a defect in the catabolism of triglyceride rich lipoproteins and the decrease in the high-density lipoprotein fraction (5, 6). Elevated triglyceride levels can indicate accumulation of triglyceride-rich, potentially atherogenic remnant particles and this finding has been reported in patients treated with chronic hemodialysis. LDL-cholesterol levels are not elevated in end-stage renal disease but changes in size and composition in association with hypertriglyceridemia could potentially result in increased atherogenicity of LDL particles (7). Recent epidemiological studies suggest, however, that a predominance of smaller and dense LDL (small, dense LDL) particles is highly atherogenic, and it may be a new risk factor for coronary heart disease. Previous results demonstrate that dyslipidemia in dialysis-treated patients includes a shift in size distribution of LDL particles toward the potentially atherogenic small LDL particles (8). Small, dense LDL is thought to be more atherogenic due to characteristics which include a lower affinity to the LDL receptor, an increased susceptibility to oxidation and an enhanced ability to penetrate to vascular intima and bind to intimal proteoglycans.

Several investigators indicate decreased lipoprotein lipase activity in renal failure (10, 12). Regarding the mechanism behind the decrease in LPL activity in uremia, three theories prevail, all of them supported by clinical or experimental findings: i) reduced enzyme synthesis by the parenchyma cell; ii) circulating inhibitor(s) against the enzyme activity and iii) heparin induced depletion of the functional enzyme pool (in hemodialysis patients).

Lipolysis of triglyceride rich lipoproteins, especially VLDL depends on the aberrant substrate characteristics of these lipoproteins. Metabolism of a lipoprotein particle is generally determined by the apoprotein composition so the disturbed apolipoprotein composition of VLDL in renal insufficiency has to be considered. There is a decrease in the apoCII/apoCIII ratio of VLDL and it is possible that it influences the substrate quality and the enzyme lipolytic reaction. Apolipoprotein CII and CIII are regulators of the LPL reaction, apo CII being the activator and apo CIII a potential inhibitor (13). In uremia, a number of potential or proven biological modifications might disturb VLDL substrate characteristics. This includes sialinization, carbamylation and glycosylation and some unidentified uremic toxins which might induce structural and functional changes in VLDL (14, 15).

Receptor-mediated uptake of lipoproteins is also disturbed and there is evidence of malfunction of the LDL (B-100/E) receptor in CRI patients and a significantly reduced catabolic rate of LDL particle. In addition to a disturbance in the LDL-receptor function, there are impairments in the receptor ligand characteristics of uremic LDL. Indeed, carbamylation and glycosylation as well as triglyceride enrichment are known to interfere with the receptor-mediated uptake of LDL. Reduced receptor-mediated uptake of LDL, for whatever reason, explains the usually reported normal LDL cholesterol concentration in spite of decreased production via the lipolytic pathway (16).

The aim of our study is to analyze disturbance in lipoprotein metabolism and the increased risk for atherosclerosis in CRI patients and those with advanced renal failure being on hemodialysis. The atherogenic risk for patients on hemodialysis is assessed by determining; total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C. Also we calculate the atherogenic risk factors that may predict the risk for cardiovascular disease because lipid abnormalities in renal diseases are associated with both a progressive decline in renal function and cardiovascular complications.

Subjects and Methods

Compositions of plasma lipid levels

Plasma lipid status was examined in three groups: CRI patients, patients on hemodialysis and a control group. The first examined group consisted of 18 CRI patients (6 females and 12 males). The second one comprised 60 patients (28 females and 32 males) on hemodialysis. Patients were dialyzed two to three times weekly for 5–6 hours using bicarbonate (BD) or acetate-based (AD) fluid on a cellulose-acetate dialysis membrane. Hemodialysis patients had received replacement therapy from one to ten years. To examine the influence of the type of dialysis on the lipid disturbance, we examined 27 patients on
acetate and 33 on bicarbonate hemodialysis. Clotting of the extracorporal circuit was avoided by using an anticoagulant such as low-molecular-weight heparin. The patients received standard heparin (1/3 as loading dose and 2/3 as continuous dose) but no other anticoagulant or anti-aggregation drugs.

Plasma lipoproteins and diabetic nephropathy. CRI patients had the following etiology: endemic nephropathy (n=7), glomerulonephritis (n=3), systemic lupus (n=1), chronic pielonephritis (n=2) and 5 were with unknown etiology. The diagnosis of chronic renal insufficiency is based on the elevated value of creatinine (>178 mmol/L). Patients on hemodialysis were with this etiology: endemic nephropathy (n=35), glomerulonephritis (n=6), systemic lupus (n=2), reflux nephropatia (n=4), bilateral nephrectomy (n=1) and 12 were with unknown etiology. Patients were dialedyzed when symptoms of uremia were detected or the value of serum urea was >35.6 mmol/L or creatinine >763 mmol/L. Patients in the predialysis and dialysis group were consuming protein-restricted diet, which is rich in carbohydrates and fat (commonly prescribed to delay the onset of uremia); none were treated with lipid-lowering drugs or hormone replacement therapy. Individuals with diabetes mellitus were excluded. The obtained results were compared with a control group of 85 (38 females and 47 males) voluntary blood donors. These individuals were matched for age and gender with the chronic renal insufficiency and dialysis group.

Sample collection

Blood samples were drawn from all patients after overnight fasting. In the subjects on hemodialysis, blood was collected prior to dialysis and heparinization. Serum lipid parameters were examined by standard biochemical methods. TC and TG concentrations were determined by standard, automated enzymatic assays using commercially available reagents (CHOD-PAP, and glycerol-3-phosphate oxidase, Randox) on the Technicon RA-X 1000. The interassay CV for TC was 1.6% and 0.9% at 3.2 mmol/L and 7.8 mmol/L, respectively. For TG the CV was 1.9% at 1 mmol/L and 1.8% at 2.2 mmol/L. HDL-C was analyzed after precipitation of non-HDL lipoproteins and subsequent determination as cholesterol in the supernatant. The interassay CV for HDL-C was 5.5% at 2.05 mmol/L and 4% at 0.98 mmol/L. LDL-C was calculated according to Friedewald formula. Atherogenic risk factors were calculated as the TC/HDL-C and LDL-C/HDL-C ratio.

Statistical analysis

Statistical evaluation was performed using the nonparametric Mann-Whitney’s U-test. The Spearman-Person test was used to evaluate the correlation between lipid parameters in the examined groups. Data were expressed as mean ± SD.

Results

Blood lipid parameters in the patients with CRI (pre-dialysis group) compared to the controls are given in Table I. Lipid disturbance is characterized by elevated TG and decreased TC, LDL-C and HDL-C concentrations. When compared to the controls, significant differences in the mean serum TG concentrations (2.41±1.09 vs. 1.40±0.80 mmol/L, p<0.001) showed. This group of patients had significantly lower mean HDL-C concentrations compared to controls (1.07±0.24 vs. 1.37±0.28 mmol/L, p<0.001). Decrease in TC and LDL-C is not significant compared to the control group (p>0.05).

Risk factors were higher for CRI patients compared to controls and there is a statistically significant difference only for the TC/HDL-C ratio (p<0.05).

Blood lipid parameters in hemodialysis patients compared to the controls are given in Table II. All lipid parameters in hemodialysis patients were statistically different compared to the controls. In hemodialysis

### Table I Plasma lipid concentrations in CRI patients compared to healthy controls (data as mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>CRI Patients (pre-dialysis group) (n=18)</th>
<th>Healthy Controls (n=85)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>49.28 ± 11.26</td>
<td>32.78 ± 19.23</td>
<td>–</td>
</tr>
<tr>
<td>Sex; Male/Female</td>
<td>12 / 6</td>
<td>47 / 38</td>
<td>–</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>5.25 ± 1.52</td>
<td>5.61 ± 1.50</td>
<td>p&gt;0.05  NS ↓</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>2.41 ± 1.09</td>
<td>1.40 ± 0.80</td>
<td>p&lt;0.001*** HS</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.07 ± 0.24</td>
<td>1.37 ± 0.28</td>
<td>p&lt;0.001*** HS ↓</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.08 ± 1.21</td>
<td>3.60 ± 1.35</td>
<td>p&gt;0.05  NS ↓</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>5.13 ± 2.10</td>
<td>4.27 ± 1.49</td>
<td>p&lt;0.05 * S ↑</td>
</tr>
<tr>
<td>LDL-C/HDL-C</td>
<td>3.04 ± 1.66</td>
<td>2.76 ± 1.24</td>
<td>p&gt;0.05  NS ↑</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. healthy controls; ** P < 0.01 vs. healthy controls; *** P < 0.001 vs. healthy controls
patients, the lipid disturbance was primarily characterized by hyper-triglyceridemia and elevated serum TG were found in more than a half of the dialyzed patients. Mean values for lipid parameters in this group indicate a decrease in TC and LDL-C concentrations and compared to healthy controls this decrease is statistically significant (p<0.05). When compared to the matched control group, hemodialysis patients had significantly reduced mean HDL-C (0.94±0.37 vs. 1.37±0.28 mmol/L, p<0.001) and significantly higher TG concentrations (2.11±1.19 vs. 1.40±0.80 mmol/L, p<0.001). All calculated ratios were higher for patients than the control group and the increases for both risk factors were statistically significant (p<0.01).

In comparison to the patients with CRI, patients on long-term hemodialysis had more severe lipid abnormalities with a significantly lower HDL-C level (0.94±0.37 vs. 1.07±0.24 mmol/L, p<0.05). All other mean values of the lipid parameters and risk ratios were lower in the dialysis group, but these differences were not significant (p>0.05).

Influence of acetate dialysis on the acid-base balance and metabolic pathways is well known, so we also tested the hypothesis of a different influence of the dialysis fluid on lipid metabolism. Patients on dialysis treatment were separated into two groups: acetate dialysis (n=27) and bicarbonate dialysis (n=33). Subjects on both types of hemodialysis also had higher TG and lower HDL-C than the control group (Table III). The obtained lipid parameters were also compared between patients on acetate dialysis and those on bicarbonate dialysis, but there was no statistical difference. Patients on bicarbonate dialysis had higher mean values of TG compared to acetate dialysis (2.27±1.26 vs. 1.90±1.08 mmol/L) and lower HDL-C (0.90±0.34 vs. 1.00±0.39 mmol/L), but there was no statistically significant difference (p = 0.14 and p = 0.22).

In Table III the results of Spearman-Pearson correlation method for all lipid parameters are presented. It should be noted that TG are positively correlated to TC and LDL-C with a statistically significant coefficient of correlation (p<0.05), but not to HDL-C. A negative correlation was found between HDL-C and most of the other lipid parameters. TG was found to be negatively correlated to HDL-C in all examined groups, and only the dialysis treated patients had a statistically significant correlation (r= 0.48, p<0.05).

<table>
<thead>
<tr>
<th>Table II</th>
<th>Plasma lipid concentrations in dialysis treated patients compared to healthy controls (data as mean ± SD).</th>
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<tbody>
<tr>
<td></td>
<td>Hemodialysis patients (n=60)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>54.48 ± 11.84</td>
</tr>
<tr>
<td>Sex; Male/Female</td>
<td>33 / 27</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.84 ± 1.23</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>2.11 ± 1.19</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>0.94 ± 0.37</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>3.00 ± 1.04</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>5.78 ± 2.30</td>
</tr>
<tr>
<td>LDL-C /HDL-C</td>
<td>3.51 ± 1.83</td>
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</tbody>
</table>
* P < 0.05 vs. healthy controls; ** P < 0.01 vs. healthy controls; *** P < 0.001 vs. healthy controls

<table>
<thead>
<tr>
<th>Table III</th>
<th>Comparison of laboratory data between two groups of CRI patients; on bicarbonate and acetate hemodialysis (data as mean±SD).</th>
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<tbody>
<tr>
<td>Acetate dialysis (n=27)</td>
<td>Bicarbonate dialysis (n=33)</td>
</tr>
<tr>
<td></td>
<td>P value</td>
</tr>
<tr>
<td>Age</td>
<td>53.37 ± 12.64</td>
</tr>
<tr>
<td>Sex; Male/Female</td>
<td>21 / 6</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>4.70 ± 1.39 **</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.90 ± 1.08 *</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.00 ± 0.39 ***</td>
</tr>
<tr>
<td>LDL-C, mmol/L</td>
<td>2.91 ± 1.28 *</td>
</tr>
<tr>
<td>TC/HDL-C</td>
<td>5.55 ± 2.43 *</td>
</tr>
<tr>
<td>LDL-C /HDL-C</td>
<td>3.34 ± 1.80</td>
</tr>
</tbody>
</table>
P < 0.05 vs. healthy controls; ** P < 0.01 vs. healthy controls; *** P < 0.001 vs. healthy controls
Lipoprotein metabolism was influenced by many determinants as the dialysis treatment itself, the use of heparin as anticoagulant, the membrane material and underlying uremia. Chronic administration of heparin induces a number of changes in the plasma lipid composition, and especially prominent was a significant rise in the plasma TG levels. A possible explanation for the heparin contribution to lipid metabolism dysfunction is the interaction of heparin with lipolytic enzymes. Heparin increases the displacement of lipoprotein lipase from its binding sites on endothelial cells and interferes in the metabolism of triglyceride-rich lipoproteins, such as VLDL. Previous studies indicated an improvement in the lipid profile of hemodialysis patients following a reduction of heparin dosage or by using low-molecular-weight heparin (25, 26). Patients were treated with low-molecular-weight heparin, and these must also be concerned in comparison with other results as a factor that improves lipid profile.

The influence of different types of dialysis fluid on lipid dysfunction could be of clinical importance. Examined patients were on acetate and bicarbonate dialysis and the obtained results for blood lipid parameters suggest the lipid abnormalities in both types of dialysis, but there was no significant difference between these two groups of patients (Table II). Influence of acetate dialysis on the acid-base balance in dialysis patients was well known and there are indications that the dialysis fluid could have effects on the lipid status of patients on chronic hemodialysis. Our data suggested that lipid abnormalities during hemodialysis are not influenced, amongst other factors, by the type of dialysis fluid.
Values for TC and LDL-C indicate lower concentrations in both groups of patients and the results obtained for patients on long-term dialysis are statistically different from the control group. Although TC and LDL-C are lower, both atherogenic risk factors TC/HDL-C and LDL-C/HDL-C are elevated in both groups of patients suggesting high risk of cardiovascular diseases development. We and other authors have reported that in patients on dialysis the TC/HDL-C ratio is abnormally high (5.78±2.30) and this ratio is higher compared to CRI patients, but not statistically significant (27).

The obtained lipoprotein profile for both patient groups, characterized with marked elevation of TG and lower HDL-C, is concerned to be very atherogenic. Although elevated LDL-C is a classical atherogenic risk factor for cardiovascular disease, new evidence suggests that even normal LDL-C can be a very atherogenic particle (8). Independently of the etiology, hypertriglyceridemia leads to prominent compositional and configurational changes of LDL-particles. Alteration in composition, size and configuration of LDL from diabetic and hemodialysis patients, impaired LDL receptor mediated degradation and these modified LDL particles are metabolized via nonsaturable scavenger receptors.

In studies on populations without renal failure, small LDL particles have been associated with hypertriglyceridemia and a low HDL-C, abnormalities characteristic of the uremic dyslipidemia and frequently linked with insulin resistance and syndrome X. Hypertriglyceridemia usually reflects triglyceride enrichment of VLDL, the natural precursor of LDL. Larger triglyceride enriched VLDL particles form a ready substrate for cholesterol ester transfer protein (CETP), which facilitates trafficking of triglyceride from VLDL to LDL in exchange for cholesterol. Thus, triglyceride content of the precursor VLDL is an important determinant of the size of the LDL (7). The resultant triglyceride-rich LDL particles are smaller and denser with alteration in the lipid-protein ratio and this leads to configuration changes of apo B which impair binding to the receptor. Small, dense LDL particles have a lower binding affinity for the apoprotein B/E receptor, resulting in longer residence times in plasma, prolonged exposure to oxidant injury and enchanted uptake by non-receptor dependent mechanisms. In addition, small, dense LDL is known to show greater susceptibility to oxidation (28, 29).

CRI patients are known from experience to have an increased risk of myocardial infarction and ischemia when compared to age and sex-matched controls (30). Lipid abnormalities probably represent one of the several potentially correctable cardiovascular risk factors associated with renal disease. Most studies in the general population have emphasized the risks associated with hypercholesterolemia, and it has been more difficult to argue the case for correction of hypertriglyceridemia, the predominant lipid abnormality associated with renal insufficiency. A change in LDL subfraction profiles associated with the accumulation of denser particles is associated with an increased risk of ischemic heart disease in non-renal populations and should be added to the list of potentially atherogenic disturbances of the lipoprotein metabolism associated with uremia (31, 32).

In conclusion, our results indicate that the prominent characteristics of lipid abnormalities in CRI patients are marked hypertriglyceridemia and low HDLC. Obtained lipoprotein profile is associated with atherogenesis and higher incidence of atherosclerotic cardiovascular complications.

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Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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