J Med Biochem 2017; 36 (4) DOI: 10.1515/jomb-2017-0019

UDK 577.1 : 61 **ISSN 1452-8258**

J Med Biochem 36: 358–365, 2017

Original paper
Originalni naučni rad

ALTERATIONS OF HDL PARTICLES IN CHILDREN WITH END-STAGE RENAL DISEASE

PROMENE U RASPODELI I FUNKCIONALNOSTI SUBFRAKCIJA LIPOPROTEINA VISOKE GUSTINE KOD DECE SA HRONIČNIM BOLESTIMA BUBREGA

Aleksandra Stefanović[1], Danijela Ristovski-Kornic[2], Jelena Kotur-Stevuljević[1], Vesna Spasojević-Kalimanovska[1], Jelena Vekić[1], Milica Miljković[1], Dusan Paripović[3], Amira Peco-Antic[3,4], Zorana Jelić-Ivanović[1], Aleksandra Zeljković[1]

[1]Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[2]Health Center Pancevo
[3]Department of Nephrology, University Children's Hospital, Belgrade, Serbia
[4]Medical Faculty, University of Belgrade, Belgrade, Serbia

Summary

Background: Unfavorable lipid profile presents one of most important risk factor for cardiovascular disease in renal pathology. Myeloperoxidase (MPO) as enzyme which oxidizes lipoproteins and paraoxonase1 (PON1) as anti-oxidative enzyme have been involved in pathogenesis of cardiovascular disease. In the present study we sought to assess oxidative stress status, lipoprotein subclasses distribution as well as functionality of high density lipoprotein (HDL) trough MPO/PON1 ratio in children with chronic kidney disease (CKD) and children after renal transplantation.
Methods: PON1 activity and oxidative stress parameters were measured spectrophotometrically, while MPO concentration was determined using immunoassay. Separation of lipoprotein subclasses was performed by vertical gradient gel electrophoresis in 19 children with different stage of CKD and 19 post-transplantation patients (PT).
Results: CKD patients had increased MPO/PON1 ratio and higher prevalence of smaller HDL subclasses when compared to PT subjects. Also, there was a significant positive correlation between MPO level and MPO/PON1 ratio with relative proportion of smaller HDL subclasses.
Conclusions: Children with CKD have impaired HDL distribution that is improved after kidney transplantation. Since that measurement of HDL distribution and functionality are

Kratak sadržaj

Uvod: Displipidemija predstavlja jedan od osnovnih faktora rizika za razvoj kardiovaskularnih bolesti kod pacijenata sa hroničnim bubrežnim bolestima (CKD). Mijeloperoksidaza (MPO) je enzim koji dovodi do oksidativne modifikacije lipoproteinskih čestica, dok sa druge strane, paraoksonaza 1 (PON 1) predstavlja antioksidativni enzim koji sprečava ove procese. Cilj ove studije bio je ispitivanje oksidativno-stresnog statusa i raspodele lipoproteinskih čestica kod dece sa različitim stadijumima CKD i nakon transplantacije bubrega. Takođe, u ovoj studiji je ispitivan odnos MPO/PON1 i njegova veza sa promenama distribucije HDL.
Metode: U studiji je učestvovalo 19 dece u različitim stadijumima CKD i 19 dece nakon transplantacije bubrega (PT). Parametri oksidativno-stresnog statusa i aktivnost enzima PON 1 određeni su spektrofotometrijskim metodama, dok je koncentracija MPO određena imunoesejem. Razdvajanje lipoproteinskih subfrakcija vršeno je metodom vertikalne gradijent gel elektoforeze.
Rezultati: U CKD grupi su dobijene statistički značajno više vrednosti odnosa MPO/PON1, kao i veći procenat malih HDL čestica u poređenju sa PT grupom. Takođe, uočena je statistički značajna pozitivna korelacija između MPO/PON1 odnosa i relativnog udela malih, HDL čestica kod ovih pacijenata.

Address for correspondence:

Aleksandra Stefanović
Department of Medical Biochemistry, Faculty of Pharmacy
Vojvode Stepe 450, P. Box 146, 11000 Belgrade, Serbia
Phone: (+381 11) 39-70-379
Fax: (+381 11) 39-72-840
e-mail: alex@pharmacy.bg.ac.rs

Non-standard abbreviations: AOPP, Advanced oxidation protein products; MPO, Myeloperoxidase; PON 1, Paraoxonase 1; SH groups, Total sulphydryl groups; TBARS, The thiobarbituric acid-reacting substances.

not routinely available, MPO/PON1 ratio may be useful marker that could provide necessary information.

Keywords: chronic kidney disease, HDL particles, myeloperoxidase

Zaključak: Rezultati ove studije pokazuju da transplantacija kod dece sa hroničnim bolestima bubrega dovodi do pomeranja raspodele HDL subfrakcija ka česticama sa bolje očuvanim funkcionalnim svojstvima. Odnos MPO/PON1 može biti koristan marker funkcionalnosti HDL.

Ključne reči: hronične bolesti bubrega, HDL čestice, mijeloperoksidaza

Introduction

Chronic kidney disease (CKD) is associated with profound changes in lipid homeostasis and therefore, dyslipidemia is one of leading co-morbidities in these patients (1). Kidney transplantation is a treatment of choice for CKD and recent data suggest that transplantation can resolve deleterious effects of kidney impairment to the lipid profile (2). However, there is a sample of evidence suggesting persistent dyslipidemia even after transplantation, mostly due to prolonged use of immunosuppressive medication (3, 4). It is important to mention that most of analyses were based on assessment of routine lipid profile markers, such as concentration of low-density (LDL) and high-density (HDL) cholesterol and triglycerides (TG), while less is known about distribution of lipoprotein subclasses in CKD patients and after transplantation, especially in younger population. Yet, children and adolescents are very vulnerable population having in mind deleterious effects of lipid disorders in early life on future cardiovascular health in adulthood (5). Therefore, advanced lipid profile analysis could be beneficial for better understanding of changes of lipid homeostasis in CKD and following renal transplantation in younger age.

In addition, contemporary research emphasize quality and resultant functionality of lipoprotein particles as far more important determinants of dyslipidemia then routinely assessed concentrations of lipid status markers. Functionality of HDL subclasses is of particular interest, because of their complex composition and their propensity toward profound changes due to alterations of other lipid, inflammatory and redox status parameters (6). However, assessment of HDL functional properties is difficult to achieve and therefore several surrogate markers are proposed. Recently, a ratio between myeloperoxidase (MPO) and paraoxonase 1 (PON1) was suggested as a competent indicator of HDL functionality (7). Giving that both MPO as a pro-oxidative and pro-inflammatory agent and PON1 as a powerful component of anti-oxidative defense system, are already recognized as important mediators of CKD-associated metabolic disorders (8, 9), it would be important to analyze their relationship in children with CKD and after renal transplantation, as well as their association with HDL subclasses distribution.

The aim of this study was to explore distribution of LDL and HDL subclasses in children and adolescents with CKD and after renal transplantation. Also, we aimed to explore levels of adipocytokines, markers of oxidative stress and anti-oxidative defense in study groups, as well as to explore their associations with HDL profile. Finally, we sought for associations between MPO, PON1 and HDL subclasses in youths with CKD and after transplantation.

Materials and Methods

Patients

For this study, we recruited 19 children and adolescent (aged 3–21) in various stages of CKD. Hemodialysis treatment was applied in 10 patients, while 9 patients were in pre-dialysis stage. Other study group was formed by 19 age and sex-matched post-transplantation (PT) patients. Post-transplantation interval was no shorter than 1 year (median: 3; interquartile range: 1–5.3 years). 79% of PT patients were previously treated by hemodialysis. All patients were selected from Nephrology Department University Children's Hospital in Belgrade. Exclusion criteria were primary cardiovascular disease, diabetes mellitus, acute infection or use of any hypolipemic medication.

Prior to the enrolment, informed consent was obtained from all participants. In case of minors, parents or tutors gave consent for involvement in the research. Entire study was planned and executed according to the ethical guidelines of the Helsinki Declaration. Local ethical committees of University Children's Hospital and Faculty of Pharmacy approved the study protocol.

At the study entry basic demographic and anthropometric data were collected. Body-mass index (BMI) was derived from weight and height, while waist and hip circumferences were used to calculate waist-to-hip ratio (WHR)

Biochemical analyses

Blood samples were taken after a 12-hour fasting period. Plasma and serum were separated by immediate centrifugation at 1500 × g for 10 minutes at 4 °C and then stored at –80 °C till analyses.

Concentrations of total cholesterol (TC) and triglycerides (TG) were measured by routine enzymatic methods using an ILab 300+ analyzer (Instrumentation Laboratory, Milan, Italy) and Randox

Laboratories (Armdore, UK) reagents. HDL-cholesterol (HDL-C) level was assessed by precipitation-method with phosphotungstic acid in the presence of magnesium ions. LDL-cholesterol (LDL-C) was determined by Friedwald's formula. Concentrations of apolipoprotein AI (apoAI) and apolipoprotein B-100 (apoB) were measured by immunoturbidimetric method using the ILab 600 analyser and Dialab (Vienna, Austria) reagents.

Oxidative stress, anti-oxidative defence and adipocytokine concentrations

The thiobarbituric acid-reacting (TBARS) concentration was measured using its molar absorption coefficient of 1.56×10^5 M^{-1} at 535 nm, previously described by Girotti (10). The intra-assay and inter-assay coefficients of variance were 4.8 % and 7.2% respectively. Concentration of advanced oxidation protein products (AOPP) were measured according to Witko-Sarsat (11), employing spectrophotometry at 340 nm. AOPP concentrations were expressed as chloramine-T equivalents. The rate of nitroblue tetrazolium (NBT) reduction was used to measure the rate of $O_2^{\cdot -}$ generation, as described by Auclair and Voisin (12) (the intra-assay and inter-assay coefficients of variance were 5.6% and 9.5%, respectively). MPO concentration was assayed by using the two-site sandwich Elisa assay (Immundiagnostik AG, Bensheim, Germany).

As a markers of antioxidative defence we measured the concentration of total sulphydryl (SH) groups in plasma and PON 1 activity. SH groups concentration in plasma was determined using 0.2 mmol/L 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) reported by Ellmann (13). DTNB reacts with aliphatic thiols (at pH 9.0) producing 1 mole of p-nitrophenol per mole of thiol. p-Nitrophenol was measured by spectrophotometry at 412 nm. PON1 paraoxon (POase activity) hydrolysis rates was measured spectrophotometrically in serum using a continuous reading spectrophotometer (Pharmacia LKB, Cambrige, UK) according to Richter and Furlong (14). The concentrations of paraoxon was 1.2 mmol/L. Paraoxon was purchased from Chem Service (West Chester, PA, USA).

Total plasma adiponectin concentration was measured in duplicate and assayed by an enzyme-linked immunosorbent assay (ELISA) method (Human Adiponectin/Acrp30 Immunoassay, Quantikine, R&D systems, Minneapolis, Minnesota, USA). The concentration of leptin in plasma was determined using a Leptin (sandwich) ELISA Kit (DRG Instruments, Marburg, Germany).

MPO/PON1 ratio was determined by subtracting MPO mass concentration by PON1 activity as previously suggested (7).

Determination of LDL and HDL subclasses

Separation of lipoprotein subclasses was performed by using the method of vertical 3–31% polyacrylamide gradient gel electrophoresis (15). After staining the gels, further analysis was accomplished using Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria) with Image Quant software (version 5.2;1999; Molecular Dynamics). Calibration curve was created based on electrophoretic mobility of Pharmacia High Molecular Weight protein standards and carboxylated polystyrene microsphere beads. Particle diameters corresponding to detected absorbance peaks were determined and each peak was classified into adequate LDL or HDL subclasses region. Assessed diameters of major peaks in LDL and HDL regions of each sample were designated as dominant LDL and HDL particle sizes. Relative proportions of individual subclasses were estimated by determination of areas under the peaks in corresponding LDL and HDL subclasses regions. Relative proportion of small, dense LDL (sdLDL) particles was determined as the fraction of the area under the peak at or below 25.5 nm in LDL region of the scan. Correspondingly, relative proportion of small HDL subclasses (HDL 3) was determined as the percentage of the area under the peak at or below 8.8 nm in HDL region of individual scan.

Statistical analysis

Normally distributed variables were expressed as means ± standard deviations (SD) and compared by the Student's t-test. TG concentration had log-normal distribution, so it was expressed or as geometrical means and the 95% confidence intervals. Asymmetrically distributed values were presented as medians and interquartile ranges and compared by the Mann-Whitney U-test. Categorical variables were shown as absolute frequencies and analysed using the Chi-square test for contingency tables.

Spearman's correlation analysis was employed to examine correlations between characteristics of HDL particles and other examined parameters. Spearman's rho value was used for screening the independent variables. We used binary logistic regression (multiple logistic regression, enter model) to seek possible independent association between increased prevalence of smaller HDL subclasses and investigated parameters which showed significant association with small HDL subclasses in correlation analysis. An increased prevalence of smaller HDL particles in an individual sample was considered if the relative proportion of HDL 3 was equal or above the upper quartile and was coded 1, while the smaller prevalence in an individual was coded 0. For each odds ratio (OR) we estimated two-tailed probability values and the 95% confidence interval (95% CI). All statistical analyses were performed using PASW Statistics version

18.0 and MedCalc Software version 11.4. Differences with $P<0.05$ were considered to be statistically significant.

Results

Basic clinical and laboratory data are presented in *Table I*. As it can be seen, both groups are age and gender matched, but CKD patients had lower BMI, although statistical significance did not reached. In addition, CKD patients had lower WHR. Other routine biochemical markers did not differ between the patients in two examined groups, but it should be noticed that concentrations of TG were markedly above the recommended values in both cohorts.

Next we analyzed advanced lipid profile and levels of adipocytokines and oxidative stress markers in CKD and PT patients (*Table II*). We found no differences in LDL particle size, as well as in relative proportion of sdLDL in both groups. In contrast, differences in HDL particles profiles between CKD and PT patients were significant. CKD patients had smaller mean HDL particle size and relative proportion of larger HDL 2b particles, alongside with increased prevalence of smaller HDL 3a, 3b and 3c subclasses. Regarding levels of adipocytokines, concentrations of leptin were comparable in the examined groups, while concentrations of adiponectin were significantly higher in CKD patients. Finally, concentrations of markers of oxidative stress and antioxidative defense were similar in both groups with an exception for SH groups and MPO, although with borderline significance. More importantly, MPO/PON1 ratio was significantly higher in CKD patients when compared to PT subjects (*Table II*).

Correlation analysis revealed significant associations of determined HDL particle characteristics with other examined markers (*Table III*). Expectedly, we found that HDL particle size was negatively correlated with TG and positively with HDL-C levels. Also, large HDL 2b subclasses were in positive and smaller HDL 3a and 3b particles in negative correlation with HDL-C concentration. In addition, proportion of HDL 3a was in positive association with TG level, but in negative with WHR. Further analysis has shown that of all markers of redox status, only activity of SOD negatively correlated with HDL size. Also, we observed positive association between adiponectin level and proportion of HDL 3b. Lastly, we found significant positive correlation of MPO level and MPO/PON1 ratio with relative proportion of smaller HDL 3a and 3b subclasses (*Table III*).

In order to further elucidate the observed relationships, we performed binary logistic regression analysis with an aim to explore independent determinants of higher presence of smaller HDL particles (*Table IV*). Binary logistic regression analysis included all variables that were associated with smaller HDL subclasses in previous consideration. As a result, only MPO/PON1 ratio was revealed as an independent predictor of higher presence of smaller HDL particles in our examinees (*Table IV*).

Table I Basic demographic and laboratoryparameters in two study groups.

Parameter	CKD patients (n=19)	PT patients (n=19)	*P*[1]
Gender (m/f)	8/11	12/7	0.194
Age (years)	14.05 ± 4.87	15.11 ± 3.26	0.439
BMI (kg/m$^{2)}$	17.86 ± 3.67	20.01 ± 3.53	0.084
WHR	0.85 ± 0.07	0.91 ± 0.08	0.046
Total protein (g/L)	65.78 ± 6.25	68.32 ± 5.76	0.207
TC (mmol/L)	4.58 ± 0.96	4.90 ± 0.94	0.319
LDL-C (mmol/L)	2.51± 0.83	2.76 ± 0.71	0.328
HDL-C (mmol/L)	1.20 ± 0.40	1.37 ± 0.54	0.284
TG* (mmol/L)	1.73 (0.45–2.56)	1.40 (0.8-2.6)	0.262
ApoAI (g/L)	1.62 ± 0.46	1.66 ± 0.45	0.815
ApoB (g/L)	0.98 ± 0.22	0.99 ± 0.25	0.903

Data are expressed as mean ± SD;
*Values are expressed as geometric means and 95th confidence intervals
[1] Continuous variables were compared using Student's t test and categorical variables by Chi-square test.

Table II Advanced lipid status profile, adipocytokines and oxidative stress parameters in analyzed groups.

Parameter	CKD patients (n=19)	PT patients (n=19)	*P*
LDL particle size (nm)	25.96±1.96	26.22±1.30	0.636
sdLDL (%)	50.62±5.41	49.42±4.10	0.448
HDL particle size (nm)	9.48±1.04	10.59±0.80	0.001
HDL 2b (%)	39.28±5.43	48.64±4.85	<0.001
HDL 2a (%)	21.27±2.13	22.58±2.28	0.077
HDL 3a (%)	16.77±2.60	14.00±2.19	<0.001
HDL 3b (%)	10.14±2.13	7.52±1.53	<0.001
HDL 3c (%)	12.56±6.52	7.17±1.49	0.002
Leptin (pg/mL)*	4.40 (2.00–11.90)	5.10 (2.10–27.90)	0.644
Adiponectin (g/mL)	26.92±14.13	18.01±11.31	0.048
SH-groups (g/L)	0.46±0.10	0.52±0.06	0.026
O_2^- (μmol/min/L)	203.43±65.16	187.23±51.39	0.401
AOPP (μmol/L) *	25.13 (18.32–31.39)	20.26 (15.83–27.62)	0.216
SOD (kU/L)	105.48± 38.11	93.85±12.91	0.221
POase (U/L) *	224.1 (183.33–346.29)	346.3 (142.59–570.36)	0.148
TBARS (μmol/L)	1.72±0.36	1.58±0.41	0.271
MPO (μg/L)*	138.05 (54.25–192.26)	61.69 (34.58–135.74)	0.086
MPO/PON1 ratio*	0.57 (0.21–0.76)	0.15 (0.07–0.60)	0.032

Data are expressed as mean ± SD and compared by Student-t test
*Values are expressed as medians and interquartile ranges and compared by Mann-Whitney U-test

Table III Correlations of HDL particle characteristics with other examined parameters in all study participants.

Parameter	HDL particle size (nm)	HDL 2b (%)	HDL 2a (%)	HDL 3a (%)	HDL 3b (%)	HDL 3c (%)
Age (years)	0.153	0.107	-0.014	0.001	-0.021	-0.118
BMI (kg/m^2)	0.120	0.151	0.293	-0.201	-0.112	-0.160
WHR	0.254	0.226	0.284	-0.351*	-0.216	-0.149
TG (mmol/L)	-0.277*	-0.168	0.246	0.320*	0.252	-0.146
HDL (mmol/L)	0.323*	0.359*	-0.270	-0.396*	-0.389*	0.005
LDL (mmol/L)	0.065	-0.055	0.192	0.044	0.112	-0.072
ApoAI (g/L)	0.253	0.216	-0.223	-0.227	-0.228	0.016
ApoB (g/L)	0.024	-0.087	0.156	0.126	0.243	-0.109
LDL particle size (nm)	0.111	0.026	0.407*	-0.077	0.042	-0.180
sdLDL (%)	0.061	-0.040	-0.250	0.002	0.080	0.112
POase (U/L)	-0.001	-0.019	0.094	-0.187	-0.274	0.190
SH-groups (g/L)	0.036	0.037	0.146	-0.165	-0.235	0.069
SOD (kU/L)	-0.354*	-0.102	0.089	0.144	-0.017	0.031
O_2^- (μmol/min/L)	-0.212	-0.194	-0.050	0.116	0.271	0.108
AOPP (μmol/L)	-0.242	-0.211	-0.098	0.236	0.303	0.066
TBARS (μmol/L)	-0.159	-0.063	0.245	0.245	0.261	-0.251
Leptin (pg/mL)#	-0.012	0.093	0.087	-0.140	-0.210	-0.114
Adiponectin (g/mL)	0.004	-0.206	-0.201	0.257	0.390**	0.058
MPO (μg/L)#	-0.251	-0.248	0.000	0.345*	0.289	0.108
MPO/PON1 ratio#	-0.241	-0.196	-0.100	0.364*	0.355*	0.137

Results are presented as Pearson's correlation coefficients.
#Results are presented as Spearman's rho coefficients due to asymmetric distribution of analyzed data; *P <0.05; ** P <0.01

Table IV Multiple logistic regression analysis for predictors of increased proportion of HDL 3 sublasses.

Predictors	OR	CI (OR)	P
MPO/PON1 ratio	5.033	1.179–21.486	0.029
TG (mmol/L)	0.463	0.091–2.360	0.354
WHR	0.000	0.000–54.891	0.120
Adiponectin (g/mL)	0.971	0.864–1.091	0.620
HDL (mmol/L)	0.457	0.091–2.360	0.690

Variables included in the model were selected according previously detected associations with HDL 3 subclasses in univariate correlation analysis. Increased proportion of HDL 3 subclasses was considered if relative proportions of HDL 3 particles were equal or above the upper quartile.

Discussion

Dyslipidemia is one of the major health concerns in patients with CKD. Lipid disorders are pivotal risk factors for development of cardiovascular complications which are the main causes of death in these patients (1). Prolonged dyslipidemia in youths with CKD could have especially detrimental effect in later adult life, therefore resolving dyslipidemia in this category of patients is of particular interest.

In this paper, we compared lipid profile in children and adolescents with CKD and after renal transplantation aiming, to determine whether transplantation leads to the improvement of lipid disorders. Regarding routine lipid markers, we found no differences between CKD and PT groups (*Table I*). Furthermore, PT patient had higher BMI and WHR, although the groups were sex and age matched. However, interpretation of anthropometric markers of adiposity in individuals with kidney impairment is difficult, due to malnutrition, inflammation and reduced muscle mass which often accompany CKD (16). Therefore, observed significantly lower WHR in CKD patients arises more likely from malnutrition-inflammation-cachexia syndrome, than it is a reflection of improved lipid profile.

Thompson et al. (2) suggested an enhancement of lipid status following renal transplantation. Our preliminary results (*Table I*) did not confirm such findings. However, more precise analysis of lipoprotein size and subclasses yielded results that are in agreement with the previous research (2). Although we did not find differences in LDL size and prevalence of sdLDL particles, we did observe significant changes in HDL size and particle distribution (*Table II*). Namely, our results clearly demonstrated enlarged HDL particle size and increased presence of larger HDL particles in PT patients. HDL particles are generally considered atheroprotective, due to their involvement in reverse cholesterol transport and their battery of anti-inflammatory, anti-oxidative and anti-thrombotic compounds (17). However, it is nowadays widely accepted that different HDL subclasses possess various protective abilities in term that in healthy individuals smaller HDL particles are the most efficient protective agents. On the other hand, dyslipidemia, oxidative stress and inflammation cause profound changes in HDL structure and function. Previous researches demonstrated that smaller HDL subclasses are particularly prone to these effects, so that they quickly lose their atheroprotective properties and may even become proatherogenic (7, 17). Having in mind that inflammation, dyslipidemia and oxidative stress are hallmarks of CKD, it is reasonable to presume that increased smaller HDL particles in this group demonstrate deficiency of atheroprotective mechanisms and may lead to accelerate atherosclerosis. In contrast, significantly reduced presence of smaller HDL particles in PT patients provides confirmation of previous findings (18) that renal transplantation could lead to improvement of dyslipidemia. It is important to mention that in previous research (18), we demonstrated less favorable HDL distribution in pediatric renal transplant recipients when compared to healthy children. Taken all together, our results imply that lipid disorders of CKD are ameliorated following transplantation, but dyslipidemia-associated cardiovascular risk should not be neglected even in post-transplantation period, particularly because of prolonged use of immunosuppressive medication.

Even it is good known that adiponectin has cardio protective and anti-inflammatory effects, the role of this adipocytokine in patients with CKD are still unclear (19). Results of previous studies have confirmed a positive association between adiponectin concentration and progression of CKD (19). The main reasons for high adiponectin concentrations in uremic patients are reduced adiponectin clearance by the impaired kidneys and changes in adiponectin binding for their receptors (20). In CKD patients, higher adiponectin concentrations which are usually more than three times higher compared with healthy individuals are associated with poor outcomes and increased mortality (21). Result of our study confirmed previous founding (19, 22) that the high levels of adiponectin decline after renal transplantation (*Table II*). Also, we found significant positive correlation between adiponectin concentration and presence of smaller HDL 3b particles (*Table III*), so we could speculate that even adiponectin is presumed to pos-

sess antiatherogenic properties in the uncommon uremic milieu, higher adiponectin levels are associated with increased risk for cardiovascular disease development. For sure, this complex role of adiponectin in CKD, needed to be investigated on deeper levels, especially in pediatric patients.

A number of studies suggest that CKD is a condition allowing a higher possibility of oxidative stress development (23). Impaired mitochondrial respiratory system could be considered both, as the consequence and the cause of increased production of reactive oxygen species (ROS), leading to damage to different intra- and extracellular structures. Our previous results showed that renal disease patients were in a state of stronger oxidative stress compared with healthy children (24). In this study, we failed to find some statistically significant differences in parameters of oxidative stress status between CKD patients and PT patients (*Table II*). This result is in agreement with previous researches, were general conclusion could be that even kidney transplantation is the ideal treatment for patients with end-stage kidney disease, the intensive oxidative stress is still one of the biggest problem for those patients and probably the major molecular mediator of adverse outcomes throughout the course of transplantation (25). Also, due to the fact that oxidative stress affect both short-term and long-term survival of PT patients different prophylactic approaches should be employed in order to reduce oxidative stress.

But, even results of our study generally didn't showed some significant changes in oxidative status parameters, very important finding of this study was markedly higher concentration of MPO in CKD patients then in PT subjects (*Table II*), although with borderline significance. It has been previously demonstrated that MPO, as a link between oxidative stress and inflammation, was elevated in CKD (25). Our results imply differences in level of MPO in CKD and after dialysis, which should be tested in larger samples. Also, it is very important to notice that, PON1 activity was higher (borderline significance) in PT patients compared with CKD (*Table II*). PON1 (aryldialkylphosphatase, EC 3.1.8.1) is a HDL-associated esterase that metabolizes oxidized lipids and has a high antioxidative potential (26). Low serum PON1 activity constitutes a risk factor for cardiovascular disease which are the leading cause of mortality in patients with CKD (27). In addition, we found significant differences in MPO/PON1 ratio which could suggest some specific perturbations in oxidative stress status in PT patients which maybe could not been seen as general improvement of oxidative status, but indicate the existence of some progress after the transplantation (*Table II*). Based on these findings, domination of pro-oxidants and possibilities of oxidative damage was more prominent in CKD than in PT patients, even though we did not observe such differences by examining other markers of redox status. More importantly, altered MPO/PON1 levels implicate disturbances in HDL structure and function, which is a complementary result with the observed changes of HDL particle distribution in CKD patients. Namely, it has been shown that MPO, PON1 and apoAI are critically important for adequate HDL function (28). Therefore, increased MPO/PON1 level founded in CKD patients completely fit to our finding of more prevalent dysfunctional smaller HDL particles in this group.

To further explore this hypothesis, we performed correlation analysis (*Table III*). Indeed, increased MPO/PON1 ratio was associated with higher relative proportions of smaller HDL 3a and 3b subclasses, which confirms previous results. Furthermore, logistic regression analysis revealed MPO/PON1 ratio as a significant and independent predictor of increased presence of smaller HDL particles (*Table IV*). Previously, Haraguchi et al. (7) proposed MPO/PON1 ratio as a useful index of impaired HDL functionality. In this study, we confirmed such findings. Although we did not explore HDL functional properties directly, we demonstrated that increased prevalence of smaller HDL particles, which are in CKD and PT individuals most likely dysfunctional, goes alongside with increased MPO/PON1 level. Since determinations of HDL distribution as well as HDL functional properties demand complex procedures which are not routinely available, MPO/PON1 ratio might be easy reachable and useful alternative that could provide necessary information.

There are several drawbacks. First, in this study we compared a group of children and adolescents with CKD with a group of renal transplant recipients. Even though the groups were age and gender matched, it would be more effective that comparison was done between the same subjects prior and after transplantation. Second, the study is performed with relatively small sample size. It is possible that some additional associations should be detected with a larger sample.

In conclusion, our results demonstrated that children and adolescents with CKD have impaired HDL distribution that is improved after kidney transplantation. MPO/PON1 ratio is strongly and independently associated with increased prevalence of smaller HDL particles, making MPO/PON1 ratio a useful marker of HDL functionality. Our findings emphasize the importance of advanced lipid testing in young patients with CKD, but also in kidney transplant recipients, which should be further explored by larger studies.

Research funding: This work is supported by grant from the Ministry of Education, Science and Technological Development, Republic of Serbia (Project numbers: 175035).

Conflict of interest statement

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

References

1. Vaziri ND. Role of dyslipidemia in impairment of energy metabolism, oxidative stress, inflammation and cardiovascular disease in chronic kidney disease. Clin Exp Nephrol 2014; 18: 265–8.
2. Thompson M, Ray U, Yu R, Hudspeth A, Smillie M, Jordan N, et al. Kidney Function as a Determinant of HDL and Triglyceride Concentrations in the Australian Population. J Clin Med 2016; 5: E35.
3. Spinelli GA, Felipe CR, Park SI, Mandia-Sampaio EL, Jr Tedesco-Silva H, Medina-Pestana JO. Lipid profile changes during the first year after kidney transplantation: risk factors and influence of the immunosuppressive drug regimen. Transplant Proc 2011; 43: 3730-7.
4. Gill JS. Cardiovascular disease in transplant recipients: current and future treatment strategies. Clin J Am Soc Nephrol 2008; 3: S29–37.
5. Halfon N, Verhoef PA, Kuo AA. Childhood antecedents to adult cardiovascular disease. Pediatr Rev 2012; 33: 51–60.
6. Kontush A, Chapman MJ. Functionally defective high-density lipoprotein: a new therapeutic target at the crossroads of dyslipidemia, inflammation, and atherosclerosis. Pharmacol Rev 2006; 58: 342–74.
7. Haraguchi Y, Toh R, Hasokawa M, Nakajima H, Honjo T, Otsui K et al. Serum myeloperoxidase/paraoxonase 1 ratio as potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease. Atherosclerosis 2014; 234: 288–94.
8. Tsai MS, Shaw HM, Li YJ, Lin MT, Lee WT, Chan KS, Myeloperoxidase in chronic kidney disease: role of visceral fat. Nephrology (Carlton) 2014; 19: 136–42.
9. Kotur-Stevuljević J, Peco-Antić A, Spasić S, Stefanović A, Paripović D, Kostić M et al. Hyperlipidemia, oxidative stress, and intima media thickness in children with chronic kidney disease. Pediatr Nephrol 2013; 28: 295–303.
10. Girotti MJ, Khan N, Mc Lellan BA. Early measurement of systemic lipid peroxidation products in plasma of major blunt trauma patients. J Trauma 1991; 31: 32–5.
11. Witko-Sarsat V, Nguyen M, Capeillere-Blandin C, Nguyen AT, Zingraff J. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49: 1304–13.
12. Auclar C, Voisin E. Nitroblue tetrazolium reduction.In: Greenworld RA, ed. CRC Handbook of Methods for Oxygen Radical research, Boca Raton, Fla: CRC Press 1985; 123–32.
13. Ellman GL. Tissue sulfhydril groups. Arc Biochem Biophys 1952; 82: 70–7.
14. Richter RJ, Furlong CE. Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics 1999; 9: 745–53.
15. Vekic J, Topic A, Zeljkovic A, Jelic-Ivanovic Z, Spasojevic-Kalimanovska V. LDL and HDL subclasses and their relationship with Framingham risk score in middle-aged Serbian population. Clin Biochem 2007; 40: 310–6.
16. Mafra D, Guebre-Egziabher F, Fouque D. Body mass index, muscle and fat in chronic kidney disease: questions about survival. Nephrol Dial Transplant 2008; 23: 2461–6.
17. Tsompanidi EM, Brinkmeier MS, Fotiadou EH, Giakoumi SM, Kypreo KE. HDL biogenesis and functions: role of HDL quality and quantity in atherosclerosis. Atherosclerosis 2010; 208: 3–9.
18. Zeljkovic A, Vekic J, Spasojevic-Kalimanovska V, Jelic-Ivanovic Z, Peco-Antic A, Kostic M et al. Characteristics of low-density and high-density lipoprotein subclasses in pediatric renal transplant recipients. Transpl Int 2011; 24: 1094–102.
19. Sopić M, Joksić J, Spasojević-Kalimanovska V, Bogavac-Stanojević N, Simić-Ogrizović S, Kravljača M, Jelić Ivanović Z. Downregulation of adipoR1 is associated with increased circulating adiponectin levels in serbian chronic kidney Disease patients. J Med Biochem 2016; 35: 436–42.
20. Jia T, Carrero J, Lindholom B, Stenvinkel P. The complex role of adiponectin in chronic kidney disease. Biochimie 2012; 94: 2150–6.
21. Matsumoto M, Ishikawa S, Kajii E. Adiponectin and noncardiovascular death: a nested case-control study. Metabolism 2008; 57: 811–8.
22. Alam A, Molnar MZ, Czira ME, Rudas A, Ujszaszi A, Klantar-Zadeh K et al. Serum adiponectin levels and mortality after kidney transplantation. Clin J Am Nephrol 2013; 8: 460–7.
23. Kao MP, Ang DS, Pall A, Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. J Hum Hypertens 2010; 24: 1–8.
24. Kotur-Stevuljevic J, Peco-Antic A, Spasic S, Stefanovic A, Paripovic D, Kostic M et al. Hyperlipidemia, oxidative stress, and intima media thickness in children with chronic kidney disease. Pediatr Nephrol 2013; 28: 295–303.
25. Cvetković T, Veličković-Radovanović R, Stojanović D, Stefanović N, Ignjatović A, Stojanović I, Sladojević N, Pavlović D. Oxidative and nitrosative stress in stable renal transplant recipients with respect to the immunosuppression protocol – differences or similarities? J Med Biochem 2015; 34: 295–303.
26. Mackness MI, Abbott C, Arrol C, PDurrington PN. The role of high-density lipoprotein and lipid-soluble antioxidant vitamins in inhibiting low-density lipoprotein oxidation. Biochem J 1993; 294: 829–34.
27. Mackness M, Durrington P, Mackness B. Paraoxonase 1 activity, concentration and genotype in cardiovascular disease. Curr Opin Lipidol 2004; 15: 399–404.
28. Schaefer EJ, Anthanont P, Asztalos BF. High-density lipoprotein metabolism, composition, function, and deficiency. Curr Opin Lipidol 2014; 25: 194–9.

Received: January 09, 2017
Accepted: March 28, 2017