

ASSOCIATION OF MYELOPEROXIDASE AND THE ATHEROGENIC INDEX OF PLASMA IN CHILDREN WITH END-STAGE RENAL DISEASE**POVEZANOST MIJELOPEROKSIDAZE I ATEROGENOG INDEKSA PLAZME KOD DECE U TERMINALNOJ FAZI BUBREŽNE INSUFICIJENCIJE**

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Summary

Background: The aim of this study was to explore oxidative stress status, especially the enzyme myeloperoxidase in children with end-stage renal disease. Also, we investigated possible associations between the atherogenic index of plasma and these parameters.

Methods: Lipid status parameters, oxidative stress status parameters, and myeloperoxidase concentration were measured in the sera of 20 children in the last stage of chronic renal disease (ESRD) and 35 healthy children of matching age and sex. The Atherogenic Index of Plasma (AIP) was calculated according to the appropriate equation.

Results: We did not find any significant differences in myeloperoxidase concentrations between the investigated groups ($p=0.394$). Oxidative stress parameters were, however, significantly higher in the patient group ($p<0.001$), as well as the atherogenic index of plasma ($p<0.001$). Myeloperoxidase concentration and advanced oxidation protein product (AOPP) concentration were independently associated with increased AIP in the patient group ($p<0.05$).

Conclusions: Changes in AIP in children with ESRD are associated with the oxidative stress status and myeloperoxidase concentration.

Keywords: myeloperoxidase, oxidative stress, chronic kidney disease

Kratak sadržaj

Uvod: Cilj ove studije bio je da se ispita status oksidativnog stresa, naročito enzima mijeloperoxidaze kod dece u terminalnoj fazi bubrežne insuficijencije. Takođe smo istraživali potencijalnu povezanost između aterogenog indeksa plazme i ovih parametara.

Metode: Parametri lipidnog statusa, parametri statusa oksidativnog stresa i koncentracija mijeloperoxidaze mereni su u serumu 20 dece u finalnoj fazi hronične bubrežne insuficijencije (HBI) i 35 zdrave dece odgovarajućeg uzrasta i pola. Aterogeni indeks plazme (AIP) izračunat je pomoću odgovarajuće jednačine.

Rezultati: Između ispitivanih grupa nisu otkrivene značajne razlike u koncentracijama mijeloperoxidaze ($p=0,394$). Međutim, parametri oksidativnog stresa bili su značajno viši u grupi obolelih ($p<0,001$) kao i aterogeni indeks plazme ($p<0,001$). Koncentracija mijeloperoxidaze i koncentracija proizvoda uznapredovale proteinske oksidacije (eng. AOPP) bili su nezavisno povezani sa povišenim AIP-om u grupi obolelih ($p<0,05$).

Žaključak: Promene u AIP-u kod dece sa HBI povezane su sa statusom oksidativnog stresa i koncentracijom mijeloperoxidaze.

Ključne reči: mijeloperoxidaza, oksidativni stres, hronična bubrežna insuficijencija

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Introduction

Cardiovascular disease is the main cause of mortality in hemodialysis patients, but the exact cause of cardiovascular damage is still unclear (1). Despite an improvement in the survival of children with severe chronic kidney disease, the risk of death due to cardiovascular disease (CVD) in children with end-stage renal disease (ESRD) is higher than in the general pediatric population (2). Emerging evidence suggests that, besides the traditional risk factors, a combination of chronic inflammation and oxidative stress may have an essential role in the development of cardiovascular abnormalities in hemodialysis (HD) patients (1–4).

It is well known that endothelial dysfunction plays a central role in the initiation of atherosclerosis (5). Oxidative modifications of low density lipoprotein particles (LDL) are among the main steps in atherosclerotic plaque formation. Evidence exists suggesting that the atherosclerotic process begins in childhood and the extent of early atherosclerosis is directly associated with the levels of lipoproteins, blood pressure, and obesity in childhood and adolescence (2). Also, it is known that hemodialysis (HD) treatment itself contributes to the increased oxidative stress in the patients and leads to chronic inflammation in ESRD (4).

Myeloperoxidase (MPO), an oxidative enzyme present in leukocyte azurophilic granules released during inflammation, causes irreversible protein and lipid modifications. Recent studies have shown that higher levels of MPO-mediated endothelial dysfunction could represent a link between oxidative stress, inflammation, and cardiovascular disease (6). However, the role of MPO in the protogenesis of chronic kidney disease (CKD), as well as its link to chronic kidney disease, though not novel (1–5), remain insufficiently investigated in the pediatric population.

In renal pathology, dyslipidemia presents one of the traditional risk factors, primarily characterized with increased triglycerides (TG) and reduced high-density lipoprotein (HDL-C) concentration. Logarithmic ratio of TG and HDL-C, known as the atherogenic index of plasma (AIP) has been successfully used as an additional index in the assessment of atherosclerosis progression (7, 8). The aim of this study was to investigate associations between AIP levels and MPO concentration, since MPO activity is an important contributor to the development of atherosclerosis. To the best of our knowledge, such associations have not been sufficiently explored.

Materials and Methods

Subjects

The study group consisted of 20 children with ESRD, all of them undergoing HD at the Hemodialysis Unit of the University Children's Hospital in

Belgrade. The control group consisted of 35 age and gender matched healthy children (*Table 1*). The study protocol included height and weight measurement for body-mass index calculation (BMI) and waist and hip measurement for waist-to-hip ratio (WHR) (*Table 1*). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at least 3 times and the presence of hypertension was defined as SBP exceeding the 95th percentile of SBP values in healthy children (9) (*Table 1*). All children and their parents gave consent prior to enrolment in our study (as required by the ethical guidelines laid down by the Declaration of Helsinki). The institutional review committees of the University Children's Hospital and the Faculty of Pharmacy, both in Belgrade, approved our study.

The HD patients were dialyzed 3.5–4 h, three times per week, with polysulfone dialyzers (Fresenius Medical Care, Bad Hamburg, Germany). Unfractionated heparin was used during HD procedures. The etiologies of underlying CKD were: congenital anomalies of the kidney and urinary tract (10), focal segmental glomerulosclerosis (4), atrophic pyelonephritis (4), and nephritic syndrome (2). HD patients had a mean duration of dialysis treatment of 3.8 ± 1.1 years. No patient received drugs that could interfere with oxidative stress. All patients were normotensive. Exclusion criteria for patients were infection, vasculitis, and respiratory or hepatic diseases.

Samples

Blood samples were obtained after overnight fasting for both controls and patients. Serum and plasma were immediately separated and stored at -80°C in aliquots until analysis.

Analytical methods

Leukocytes were determined by a combination of flow impedance and light absorbance using a Pentra 60C+ analyzer (Horiba ABX, Montpellier, France).

High sensitivity serum CRP (hsCRP) levels were measured by the latex-enhanced immunoturbidimetric method (Quantex hsCRP kit, BLOKIT, Barcelona, Spain) on an ILab 600 analyzer (Instrumentation Laboratory, Milan, Italy). Fibrinogen was measured in citrate plasma using the Clauss method on an ACL 200 (Instrumentation Laboratory) using original reagents (10).

Total cholesterol (TC) and TG were analyzed by routine enzymatic methods using an ILab 300+ analyzer (Instrumentation Laboratory, Milan, Italy) and Randox Laboratories (Armdore, UK) reagents. HDL-C was measured using the same method by precipitating plasma with phosphotungstic acid in the presence of magnesium ions. Apolipoprotein A-I (apoA-I) and apo-

lipoprotein B (apoB) were measured by immunoturbidimetry using an ILab 600 analyzer and Dialab (Vienna, Austria) reagents. The concentration of LDL cholesterol (LDL-C) was calculated using the original equation of the Friedewald formula (11).

In order to determine the level of lipid peroxidation, we measured MDA plasma concentration as thiobarbituric acid-reacting substances using the method previously described by Girotti (12). The rate of nitro blue tetrazolium reduction was used to measure the plasma level of superoxide anion ($O_2^{\cdot-}$) production as a marker of oxidative stress using the method of Auclair and Voisin (13). Plasma superoxide dismutase (SOD) activity was measured according to the method published by Misra and Fridovich (14). One unit of SOD activity is defined as the activity that inhibits the autooxidation of adrenalin by 50%. The plasma advanced oxidation protein products (AOPP) concentration was determined using the method of Witko-Sarsat et al. (15). Briefly, AOPP were measured in acidic conditions at 340 nm in the presence of potassium iodide and the concentration was expressed as chloramine-T equivalents (μmol Hloramin T Equiv./L) (15). Determination of serum paraoxonase 1 (PON1) status involved measurement of PON1 activity towards two substrates: paraoxon (POase activity) and diazoxon (DZOase activity). Paraoxon PON1 and diazoxon hydrolysis rates were measured spectrophotometrically in serum using a continuous reading spectrophotometer (Pharmacia LKB, Cambridge, UK) according to Richter and Furlong (16). Population distribution plots of diazoxonase versus paraoxonase activities provide PON1 phenotypes: PON1192QQ, PON1192QR, and PON1192RR (16). PON1 Q192R polymorphism affects the catalytic efficiency of PON1 towards some of its substrates: PON1192QQ isoform hydrolyzes diazoxon rapidly as compared to R192 isoform, while PON1192RR isoform hydrolyzes paraoxon rapidly as compared to PON1 Q192 isoform (16).

MPO concentration was assayed by using the two-site sandwich Elisa assay (Immundiagnostik AG, Bensheim, Germany), following the manufacturer's instructions. The absorbance was measured using an ELISA reader (Pharmacia LKB, Cambridge, UK) at 450 nm against 620 nm as a reference.

The Atherogenic Index of Plasma (AIP) was calculated according to the following equation: $AIP = \log(TG/HDL-C)$, with units for TG and HDL-C in mmol/L (8).

Statistical analysis

Values were expressed in two ways, either as means \pm standard deviations (SD) for normally distributed variables or as geometrical means and the 95% confidence intervals if distribution was log-normal. Comparisons of continuous variables were per-

formed using the Student's t-test. Categorical variables are shown as relative or absolute frequencies. Analysis of categorical variables was performed using the Chi-square test for contingency tables.

To estimate the correlation between the examined parameters of lipid profile and oxidative stress and AIP, we performed Spearman's correlation analysis. Spearman's rho correlation test was used for screening the independent variables. If P-values for Spearman's rho coefficient of correlation were < 0.10 , the variables were included in further regression analyses. We used univariate regression analysis to estimate significant independent association of the investigated parameters with AIP. Further statistical analysis referred to multiple regression analysis. The tolerance option was used to prevent multicollinearity among the independent variables (17). All statistical analyses were performed using PASW Statistics version 18.0 and MedCalc Software version 11.4. All statistical tests were considered significant at the 0.05 probability level.

Results

General demographic and anthropometric parameters are presented in *Table I*. We have noticed significantly higher waist-hip-ratio (WHR), as well as SBP and DBP in patients than in healthy children, while BMI value was significantly lower in the patient group (*Table I*).

Biochemical parameters, oxidative stress and antioxidative defense parameters in both children with ESRD (patients) and healthy children are presented in *Table II*. Concentrations of TG and total WBC count were significantly higher in the group of patients, while concentrations of HDL-C and LDL-C were significantly lower compared with healthy children (*Table II*). Also, we found higher parameters of oxidative stress, AOPP and MDA, in the patient group. Both PON1 enzyme activities, DOase and POase, were lower in the patient group, but only POase was significantly lower. Furthermore, the difference was statistically significant only for POase activities (*Table II*). As expected, both SOD activity and AIP were significantly higher in the patient group (*Table II*).

Table III shows Spearman's non-parametric correlations between AIP and biochemical parameters, as well as the parameters of oxidative stress in HD and healthy children.

If P-values for Spearman's rho coefficient of correlation were less than 0.1, the variables were considered for further regression analysis. BMI, DBP, AOPP and MPO concentrations all have a significantly positive correlation with AIP. Only MPO showed a positive correlation in both healthy children and the patient group (*Table III*).

In order to avoid multicollinearity and to provide the correct model for parameters investigation, we per-

Table I Demographic and anthropometric data in patients and control group.

| Parameter | Controls (n=35) | Patients (n=20) | <i>p</i> ¹ |
|------------------------------|-----------------|-----------------|-----------------------|
| Gender (m/f) | 19/16 | 9/11 | 0.702 |
| Age (years) | 12.2±2.51 | 12.7±5.72 | 0.659 |
| BMI (kg/m ²) | 18.8±3.33 | 16.5±2.85 | < 0.05 |
| WHR | 0.8±0.04 | 0.9±0.06 | < 0.001 |
| Duration of dialysis (years) | - | 3.8±1.1 | - |
| SBP (mmHg) | 112.0±9.2 | 127.2±22.3 | < 0.001 |
| DBP (mmHg) | 71.3±7.4 | 78.2±20.1 | < 0.05 |

Data are expressed as mean ± SD; *- means and 95th confidence intervals derived from log-normal values

¹ Continuous variables were compared using Student's t test and categorical variables by Chi-square test.

BMI – Body Mass Index; WHR – Waist to Hip Ratio; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure

Table II Clinical and laboratory parameters, OS/antioxidative defense parameters, PON1 activities and PON1 phenotypes distribution in the two study groups.

| Parameter | Controls (n=35) | Patients (n=20) | <i>p</i> ¹ |
|--|-------------------|-------------------|-----------------------|
| TC (mmol/L) | 4.5±0.84 | 4.2±1.18 | 0.344 |
| LDL-C (mmol/L) | 2.7±0.63 | 2.2±0.65 | < 0.05 |
| HDL-C (mmol/L) | 1.4±0.35 | 1.2±0.52 | < 0.05 |
| TG* (mmol/L) | 0.7 (0.602–0.801) | 1.7 (1.381–2.177) | <0.001 |
| AIP | -0.3±0.188 | 0.2±0.307 | < 0.001 |
| Total protein (g/L) | 69.8±4.06 | 63.6±7.54 | < 0.001 |
| WBC (10 ³ /mm ³) | 6.8±1.63 | 8.2±3.39 | < 0.05 |
| MPO (µg/L) | 57.1±15.44 | 66.6±6.31 | 0.394 |
| MDA (µmol/L) | 1.2±0.39 | 1.8±0.34 | < 0.001 |
| O ₂ ⁻ (µmol/min/L) | 164.5±69.52 | 156.3±83.22 | 0.701 |
| AOPP (µmol/L) | 12.4±3.84 | 27.9±41.9 | < 0.001 |
| SOD (kU/L) | 104.9±28.6 | 128.1±35.61 | < 0.05 |
| SH-groups (g/L) | 0.5±0.05 | 0.5±0.13 | 0.827 |
| POase (U/L) | 435.2±91.91 | 232.2±86.53 | < 0.05 |
| DZOase (U/L) | 10581±4081 | 9354.9±2337.5 | 0.372 |
| PON1 phenotype, (%) | | | 0.936 |
| QQ | 59% | 57% | |
| QR | 26% | 28% | |
| RR | 15% | 15% | |

Data are expressed as mean ± SD; *- means and 95th confidence intervals derived from log-normal values

¹ Continuous variable were compared using Student's t test and categorical variables by Chi-square test.

TC – Total Cholesterol; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; TG – Triglycerides; AIP – Atherogenic Index of Plasma; WBC – White Blood Cell; MPO – Myeloperoxidase; AOPP – Advanced Oxidation Protein Products; SOD – Superoxide Dismutase; SH-groups – Sulfhydryl groups; POase – Paraoxonase activity; DZOase – Diazoxonase activity

Table III Spearman's non-parametric correlations between AIP and biochemical and oxidative status parameters for the two study groups.

| Parameter | Controls | | Patients | |
|--|----------|-----------------|----------|------------------------------|
| | ρ | P | ρ | P |
| BMI (kg/m ²) | -0.09 | 0.607 | 0.467 | <0.05[#] |
| WHR | 0.240 | 0.164 | -0.182 | 0.470 |
| SBP (mmHg) | -0.328 | 0.082 | 0.422 | 0.064 [#] |
| DBP (mmHg) | -0.169 | 0.309 | 0.540 | <0.05[#] |
| Glucose (mmol/L) | 0.023 | 0.895 | 0.122 | 0.609 |
| TC (mmol/L) | 0.076 | 0.666 | 0.160 | 0.499 |
| LDL-C (mmol/L) | 0.142 | 0.417 | 0.007 | 0.977 |
| MPO (μ g/L) | 0.360 | <0.05 | 0.595 | <0.001[#] |
| MDA (μ mol/L) | 0.197 | 0.287 | 0.394 | 0.096 [#] |
| O ₂ ⁻ (μ mol/min/L) | -0.307 | 0.102 | 0.433 | 0.064 [#] |
| AOPP (μ mol/L) | -0.397 | 0.057 | 0.744 | <0.001[#] |
| SOD (kU/L) | -0.085 | 0.634 | -0.019 | 0.937 |
| SH-groups (g/L) | -0.391 | 0.385 | -0.307 | 0.265 |
| POase (U/L) | -0.179 | 0.320 | -0.067 | 0.853 |
| DZOase (U/L) | 0.126 | 0.486 | 0.515 | 0.128 [#] |

[#]Variables with $p < 0.10$ were entered in multiple logistic regression analysis

BMI – Body Mass Index; WHR – Waist to Hip Ratio; TC – Total Cholesterol; LDL – Low Density Lipoprotein; HDL – High Density Lipoprotein; TG – Triglycerides; AIP – Atherogenic Index of Plasma; SBP – Systolic Blood Pressure; DBP – Diastolic Blood Pressure; WBC – White Blood Cell; MPO – Myeloperoxidase; AOPP – Advanced Oxidation Protein Products; SOD – Superoxide Dismutase; SH-groups – Sulfhydryl groups; POase – Paraoxonase activity; DZOase – Diazoxonase activity

Table IV Linear regression analysis for the association of investigated parameters with the Atherogenic Index of Plasma (AIP) in patients (univariate analyses).

| | β | SE (β) | P | R ² | adjusted R ² |
|--|---------|----------------|------------------|----------------|-------------------------|
| MPO (μ g/L) | 0.515 | 0.001 | <0.05 | 0.266 | 0.225 |
| MDA (μ mol/L) | 0.407 | 0.198 | 0.084 | 0.165 | 0.116 |
| O ₂ ⁻ (μ mol/min/L) | 0.469 | 0.001 | 0.043 | 0.220 | 0.174 |
| AOPP (μ mol/L) | 0.691 | 0.003 | <0.001 | 0.478 | 0.447 |

Table V Multiple regression analysis for the association of investigated parameters with the Atherogenic Index of Plasma (AIP) in patients.

| AIP R ² = 0.532 adjusted R ² = 0.365 | | | | |
|--|---------|----------------|-----------------|--|
| | β | SE (β) | P | |
| BMI (kg/m ²) | 0.243 | 0.025 | 0.320 | |
| Dialysis duration (months) | 0.089 | 0.002 | 0.702 | |
| SBP (mmHg) | 0.087 | 0.004 | 0.781 | |
| DBP (mmHg) | -0.264 | 0.005 | 0.425 | |
| MPO (μ g/L) | 0.503 | 0.001 | <0.05 | |

Table VI Multiple regression analysis for the association of investigated parameters with the Atherogenic Index of Plasma (AIP) in patients.

| AIP R ² = 0.679 adjusted R ² = 0.555 | | | |
|--|--------|--------|--------------|
| | β | SE (β) | P |
| BMI (kg/m ²) | 0.266 | 0.021 | 0.582 |
| Dialysis duration (months) | -0.121 | 0.002 | 0.567 |
| SBP (mmHg) | 0.038 | 0.004 | 0.888 |
| DBP (mmHg) | -0.266 | 0.004 | 0.354 |
| AOPP (μmol/L) | 0.658 | 0.003 | 0.002 |

formed a univariate regression analysis in the patient group (Table IV). MPO and AOPP concentrations showed independent association with AIP (Table IV).

In order to further investigate the independent influence of MPO and AOPP on AIP, we performed a multiple regression analysis. We made two models, one for MPO investigation and another for AOPP (Table V, Table VI). In accordance with the results of Spearman's correlation analysis, we included BMI, duration of dialysis, SBP, DBP in both models (Table IV, Table VI).

The obtained results confirmed our prediction. MPO and AOPP concentrations were independently associated with increased AIP (Table V, Table VI). In fact, 36.5% of the AIP increase in patients could be explained by MPO and even 55.5% of AIP changes could be explained by the increase in AOPP concentrations in the patients.

Discussion

It is well known that pediatric renal diseases are associated with a significant rise in CVD risk (3). Prevention of atherosclerosis and CVD in the pediatric population is of particular importance because children with chronic kidney disease are an extremely vulnerable group. Therefore, the discovery of a new reliable marker for early detection of the origin and development of CVD risk in ESRD patients is of great medical importance.

Dyslipidemia, including increased LDL-C, TG and decreased HDL-C and ApoA1 levels, in ESRD is the most important risk factor for atherosclerosis (18, 19). Expectedly, elements of dyslipidemia were also present in our patient group (Table II). Generally, dyslipidemia develops as a consequence of unbalanced energy metabolism and adiposity. We have shown differences in the markers of adiposity between our examinees. BMI was significantly reduced, while WHR was increased in the group of patients (Table I).

In people with established renal disease, there is controversy regarding the parameter which would most accurately reflect adiposity. Evaluation of BMI in CKD has yielded conflicting results (20, 21). Children with kidney diseases may have lower BMI due to reduction of the muscle mass which is associated with greater cardiovascular risk. According to INTERHEART study, WHR has stronger predictive power for cardiovascular events than BMI (20). Accordingly, it has been shown that in children on HD, normal or low BMI and elevated WHR may indicate a greater risk of cardiovascular disease development (22), and such findings are consistent with our results (Table I). BMI in our patients was even lower than in control group, but elevated WHR pointed toward increased CVD risk.

Beside traditional risk factors such as hypertension and dyslipidemia, OS may play an important role in the pathogenesis of CVD (3). Hemodialysis represents a therapeutic procedure in patients with renal impairment, but it is believed that dialysis leads to chronic inflammation caused by the activation of phagocytes. Hemobioincompatibility depends on the nature of the dialysis membrane, as well as of the possible endotoxin contamination of dialysate. Activated neutrophils and monocytes lead to the formation of reactive intermediates which contribute to the oxidative unbalance and develop oxidative stress in which myeloperoxidase plays an important role (23).

Therefore, it has been proposed that HD can be considered a human model of OS. Our results fit in such a hypothesis, since our HD patients exhibited significantly higher OS, as reflected by elevated MDA and AOPP levels. Witko-Sarsat et al. (15) investigated a link between MPO and monocyte activation in patients with chronic renal failure. They came to the conclusion that the AOPP are capable of releasing inflammatory mediators and thus represent a new class of inflammatory mediators and markers of oxidative stress (15).

Conditions that are accompanied by OS and inflammation are usually associated with reduced activity of paraoxonase (PON1). PON1 is an antioxidant enzyme located on the HDL particle (24). In addition to its antioxidant role, PON1 shows anti-atherogenic properties by preventing the formation of foam cells, through reducing the influx of cholesterol and oxidized lipids and inhibiting macrophage cholesterol synthesis (25). PON1 activity is reduced in patients with renal diseases. Although some studies showed the benefits of HD via increasing the anti-inflammatory activity of HDL, other researches showed no differences in PON1 related to HD. Furthermore, several studies even demonstrated reduction in PON1 activity in patients on HD (24, 26). Our results showed lower PON1 activity in children on HD as compared with controls (*Table II*). Lower POase activity of PON1 in nephrology patients could arise as a result of partial inactivation of the enzyme due to increased oxidative stress, which was evident among the patients in our study (*Table II*).

Surprisingly, we found no differences in MPO concentrations between children on HD and their healthy counterparts (*Table II*). So far, little data has been reported on the changes of MPO levels in children on HD. Lack of significant differences between the healthy and HD groups in our study could be a consequence of the relatively small sample size, but also of the younger age of our examinees and lower intensity of inflammation, as demonstrated by the total WCB count (*Table II*).

Next, we examined associations of changes in lipid profile and oxidative stress in children on HD treatment with AIP. Previously, AIP was proposed as a new possible marker that can be used for detection of early atherosclerosis. In recent years, this parameter has started to gain importance as an indicator of atherosclerosis (27, 28). It has been suggested that AIP is less susceptible to variations in disease activity during longer periods of time. This makes it more attractive for use in cardiovascular risk prediction than lipid concentrations *per se*, especially in children with ESRD (8). Elevated AIP is connected with higher percent of remnant lipoproteins and small, dense LDL (sdLDL) particles (29, 30). The impaired HDL composition, increased serum levels of remnant lipoproteins, reduced levels of very low density and intermediate density lipoproteins, and increased sdLDL are the features of an atherogenic lipid profile in children with ESRD (30). When compared to larger LDL subclasses, sdLDL particles are easily taken up by arterial tissue and are much more prone to oxidation which altogether can lead to accelerated plaque formation. Therefore, elevated AIP might reflect the presence of remnant lipoproteins and sdLDL levels (30). Also, in our research, AIP was in significant positive correlation with MPO in both controls and patient group (*Table III*), reflecting the cooperative effects of dyslipidemia, OS, and inflammation in the onset and pro-

gression of atherosclerosis. Previous studies have demonstrated that HD treatment increases the production of MPO from azurophilic granules. Also, it has been shown that MPO prolongs the life span of neutrophils by suppressing the constitutive cell death program. In this way, MPO may extend inflammation (31, 32). Additionally, the revealed independent associations between MPO and AIP in our study (*Table V*) raised a possibility of more profound involvement of MPO in the metabolic alterations of lipoprotein distribution.

AIP is a simple and practical ratio which can be easily estimated and used to further stratify atherogenic risk in subjects who may have an apparently normal lipid profile according to other parameters. Having in mind the simplicity of determining AIP, we further tried to identify the pattern and predictors of an abnormal AIP in children on HD. According to our results, the AIP correlated with BMI, DBP, AOPP, and MPO (*Table III*). Stepwise multiple linear regression analysis was carried out to determine the significant predictors of AIP. We found that MPO and AOPP are independently associated with AIP (*Table IV, Table V*, respectively). On the basis of the foregoing facts, we hypothesize that AIP should be routinely analyzed from the beginning of HD treatment because it can indirectly reflect the degree of oxidative stress caused by the release of MPO and the resulting increase in AOPP. Our results have shown that the AIP level is not dependent on the duration of HD; however, it is strongly influenced by the oxidative enzyme MPO and its products.

In conclusion, our results demonstrated elevated OS, inflammation, and patterns of dyslipidemia in children with ESRD, indicating elevated CVD risk in this group. AIP was independently associated with MPO and AOPP, suggesting firm connections between lipid disorders, inflammation, and OS in this category of patients. Also, AIP emerged not only as a hallmark of dyslipidemia, but also as a quickly accessible marker of the interplay between several pathophysiological processes involved in the elevation of CVD risk in children with ESRD. Future cohort studies are needed to evaluate our present findings.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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