Clinical Study

Asymmetric and Symmetric Dimethylarginine in Adolescents with Hyperuricemia

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The purpose of this work was to investigate if in adolescents with hyperuricemia serum levels of asymmetric and symmetric dimethylarginine (ADMA, SDMA) are increased and if their levels correlate with serum uric acid (UA). *Patients and Methods.* The study group consisted of 58 hyperuricemic patients aged median 16.15 Q1–Q3 (14–17). The reference group contained 27 healthy individuals with normal serum UA level. ADMA and SDMA were measured by immunoenzymatic ELISA commercial kits and expressed in $\mu\text{mol/L}$. Serum UA was measured by the colorimetric method. *Results.* In hyperuricemic patients serum ADMA values did not differ between two estimated groups ($P > 0.05$); however, SDMA was significantly higher than in reference group ($P < 0.01$). Serum ADMA and SDMA correlated positively with UA ($r = 0.34$, $P < 0.01$) ($r = 0.31$, $P < 0.01$) and hs-CRP ($r = 0.20$, $P < 0.05$) ($r = 0.36$, $P < 0.01$), respectively. *Conclusion.* We demonstrated increased SDMA but not ADMA levels in adolescents with hyperuricemia and their correlation with serum uric acid levels. However, at the moment it is difficult to answer the question if it is just coexistence of these factors or any mechanism linking uric acid and methylated arginines really exists.

1. Introduction

Uric acid (UA) has been formerly considered as a major antioxidant in human plasma with possible beneficial antiatherosclerotic effects; however, since the beginning of the Twentieth century, uric acid has been suggested as a risk factor for cardiovascular diseases [1]. While epidemiologic studies suggested that hyperuricemia (HU) is strongly correlated with cardiovascular disease, it is still unclear whether hyperuricemia is an independent risk factor of cardiovascular disease [2]. Some clinical evidence has found a significant and specific association between serum uric acid levels and coronary atherosclerosis; however, there is much controversy concerning this relationship, and some studies in fact came to the opposite conclusions [2, 3]. High uric acid levels could potentially increase the risk of cardiovascular disease via several biological mechanisms; however, recent studies have suggested that endothelial dysfunction is a fundamental mechanism whereby uric acid may affect cardiovascular

function. Series of animal experiments revealed that in rats, hyperuricemia induced by oxonic acid, a uricase inhibitor, causes hypertension and renal arteriopathy and impairs nitric oxide generation [4]. Moreover, oxidative stress plays an important role in the vascular endothelial dysfunction of hyperuricemia [4, 5].

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor [6]. An increase in the levels of ADMA is likely to be a factor connected with impaired nitric oxide production and resulting in endothelial dysfunction in cardiovascular disease, including hypertension, atherosclerosis, and metabolic syndrome [7]. It was confirmed that ADMA concentrations were elevated in patients with cardiovascular disease [8]. Recent studies suggested ADMA to be a sensitive marker and even an initiator of endothelial dysfunction [9].

Similarly, the second form-symmetric dimethylarginine (SDMA) has insignificant inhibitory effects on nitric oxide synthase. However, it has recently been suggested that SDMA

might have an indirect effect on nitric oxide synthesis via inhibiting the transporter that mediates the intracellular uptake and absorption of L-arginine [10].

Although previous studies suggested that serum uric acid as well as ADMA and SDMA levels might correlate with cardiovascular risk or renal disease, the relationship between serum uric acid and ADMA or SDMA has not yet been characterized.

The goal of this work was to investigate if in adolescents with hyperuricemia serum levels of ADMA and SDMA are increased and if their levels correlate with serum uric acid. Additionally we would like to check if serum level of any of these dimethylarginines correlates with hs-CRP.

2. Patients and Methods

The protocol was approved by the Bioethics Committee of the Medical University of Białystok in accordance with the Declaration of Helsinki. Informed consent was obtained from parents of all participants and children older than 16 years of age.

Fifty-eight patients aged median 16.15 Q1–Q3 (14–17). (M—35; F—23) with elevated serum uric acid were eligible to participate in the study. Patients were recruited from referrals to Department of Pediatric Nephrology, Medical University of Białystok, from June 2010 until May 2012. The reason for hospitalization was elevated causal blood pressure in the General Practitioner Office. Reference group (R) consisted of 27 healthy children (12 boys, 15 girls; aged median 15.8 Q1–Q3 (10.8–17.1)) with normal serum uric acid levels.

Patients who meet all the following inclusion criteria were enrolled into the study: (1) age 10–18 years, (2) hyperuricemia defined as serum uric acid above 5.5 mg/dL [11], (3) normal renal function (normal creatinine level, eGFR > 90 mL/min/1.73 m², no proteinuria), (4) no clinical and laboratory signs of infection, (5) not treated with antibiotics within the last 4 weeks, and (6) signed the informed consent.

Patients with a history of gouty arthritis, renal stones, protein/creatinine ratio >0.2, and diabetes mellitus were excluded. In all adolescents, careful clinical history, underlying comorbidities, and physical examination were assessed. Body weight and height were measured using a balance beam scale and pediatric wall-mounted stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m²). BMI Z-scores, which reflect the SD score for age- and gender-appropriate BMI distribution, were calculated using the following formula: $Z = [X - \mu]/\sigma$; X is the BMI measured in the patient, whereas μ and σ represent the mean and the standard deviation for age- and gender-matched controls [12]. Based on the international norms from the WHO [13] with age- (to the nearest one month) and gender-specific BMI, BMI cutoffs were the following: overweight—BMI > +1SD; obesity—BMI > +2SD. Hypertension (HT) on the basis of ABPM was defined as mean systolic or diastolic daytime or nighttime BP levels that are ≥95th percentile. Ambulatory blood pressure monitoring (ABPM) was performed using the oscillometric SpaceLab device (Spacelab CardioNavigator). The monitors were programmed to measure BP every 15 minutes during

daytime (8:00–22:00) and every 30 minutes during nighttime (22:00–8:00); however, the periods were corrected according to the patients' diary. Recording started between 8 and 9 am and lasted for 24 hours. Recordings with a minimum 80% of measurement and without breaks longer than 2 hours were considered sufficient.

After an overnight fast, five milliliters of venous, peripheral blood was collected from each patient and reference participants during routine laboratory testing. Isolated serum aliquots were stored at –80°C for further analysis. The biochemical workup included serum creatinine (measured by Jaffe reaction), urea, fasting plasma glucose (FPG), lipid profile, and serum uric acid concentration. The estimated glomerular filtration rate (eGFR) was calculated from the updated Schwartz formula: $\text{GFR (mL/min/1.73 m}^2\text{)} = (0.41 \times \text{Height in cm})/\text{Creatinine in mg/dL}$. ADMA and SDMA were measured in serum using ELISA method (Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's guidelines. Serum ADMA and SDMA levels were expressed in $\mu\text{mol/L}$. The intra- and interassay coefficients of variance (CVs) for ADMA were 6.5–7.0% and 6–7% and for SDMA 4.8–7.5% and 6–7%, respectively.

Serum hs-CRP was assessed by particle-enhanced immunonephelometry using a standard CardioPhase hs-CRP for BNII (Dade Behring Diagnostics, Marburg, Germany) and expressed in mg/L. The intra-assay and interassay coefficient of variation for hs-CRP were 4.2% and 6.8%, respectively.

2.1. Statistical Analysis. All continuous variables were tested for normal distribution by the Kolmogorov-Smirnov, with Lilliefors correction and Shapiro-Wilk tests. As most of the studied parameters were not normally distributed, descriptive statistics were calculated as median with the interquartile range. Mann-Whitney U test was used to compare continuous variables, and χ^2 -test with Yates correction was used to compare categorical variables between two groups. The correlations between SDMA, ADMA, and other studied variables were assessed using Spearman correlation test. Two multiple linear regression models were created including the SDMA and ADMA as dependent variables. Statistical significance was determined at $P < 0.05$ level. All calculations were made using the Statistica 10.0 program (StatSoft Inc., USA).

3. Results

The clinical and biochemical characteristics of the study participants are listed in Table 1. Overall median age of all children with hyperuricemia was 16.15 Q1–Q3 (14–17) yrs. The age and sex of studied children did not differ from healthy controls ($P > 0.05$). Almost 58% of teenagers with hyperuricemia were overweight or obese and 60% were hypertensive. Median BMI Z-score in both examined groups was 1.08 (Q1–Q3: –0.36–1.64) in studied children and 0.2 (Q1–Q3: –0.2–0.8) in reference group ($P < 0.01$). Total cholesterol values were significantly higher in studied patients (median: 162 mg/dL) than in reference group (median: 142 mg/dL; $P < 0.05$). No significant differences

TABLE 1: Clinical and biochemical characteristics of patients and the reference group.

Variables	Patients	Controls	P
	Median (Q1–Q3)		
Age (years)	16.15 (14–17)	15.8 (10.8–17.1)	NS
BMI (Z-score)	1.08 (–0.36–1.64)	0.2 (–0.2–0.8)	<0.01
Serum creatinine (mg/dL)	0.74 (0.57–0.86)	0.57 (0.43–0.71)	<0.01
Serum urea (mg/dL)	26 (23–30)	22.5 (20–27)	<0.05
Serum uric acid (UA) (mg/dL)	6.5 (5.83–7.13)	4.06 (3.3–4.7)	<0.01
eGFR (mL/min./1.73 m ²)	99.76 (88.41–121.45)	117.72 (111.43–144.05)	<0.01
Fasting plasma glucose (FPG) (mg/dL)	91.5 (87–95)	91 (88–93)	NS
Triglycerides (TG) (mg/dL)	93 (76–138)	85 (61–117)	NS
Total cholesterol (mg/dL)	162 (145–187)	142 (136–166)	<0.05
HDL (mg/dL)	52.36 (42.00–57.00)	55.00 (52.36–56.00)	NS
LDL (mg/dL)	90.66 (73.00–104.00)	90.00 (78–90.66)	NS
ADMA (μmol/L)	0.47 (0.41–0.53)	0.46 (0.83–0.49)	NS
SDMA (μmol/L)	0.54 (0.44–0.63)	0.51 (0.39–0.53)	<0.01
hs-CRP (mg/L)	0.74 (0.17–3.52)	0.17 (0.13–1.54)	<0.01
Albuminuria (mg/24 h)	4.55 (2.72–9.9)	5.95 (0.28–100.8)	NS
SBP (mmHg)			
D	136 (130–140)	118 (111–124)	<0.01
N	120 (115–124)	110 (105–112)	<0.01
DBP (mmHg)			
D	73 (69–78)	67 (64–70)	<0.01
N	64 (60–67)	57.5 (54–59)	<0.01

FIGURE 1: Correlation between ADMA, SDMA, and biochemical parameters in hyperuricemic patients.

between those two groups were observed regarding FPG, triglycerides, HDL, and LDL.

When compared to healthy controls, hyperuricemic patients showed increased serum SDMA ($P < 0.01$), but not serum ADMA levels (Table 1).

Serum ADMA and SDMA correlated positively with UA ($r = 0.34$, $P < 0.01$) ($r = 0.31$, $P < 0.01$), respectively, and

hs-CRP ($r = 0.20$, $P < 0.05$) ($r = 0.36$, $P < 0.01$), respectively (Figure 1). The positive correlation was observed between serum ADMA and BMI Z-score ($r = 0.36$, $P < 0.01$), TG ($r = 0.46$, $P < 0.01$), total cholesterol level ($r = 0.28$, $P < 0.01$), and LDL-cholesterol (LDL-C) ($r = 0.33$, $P < 0.01$) in hyperuricemic patients. Additionally SDMA correlated positively with creatinine level ($r = 0.27$, $P < 0.01$)

TABLE 2: Results of linear regression for ADMA and SDMA.

	R^2	Stand. B	SE	P value
Predictors of ADMA	0.374			
Serum uric acid (UA)		0.016	0.0069	0.02
TG		0.0009	0.0001	<0.01
LDL-C		0.002	0.0009	0.01
Predictors of SDMA	0.214			
Serum uric acid (UA)		0.012	0.0069	0.05
eGFR		-0.05	0.0001	0.04
Hs-CRP		0.07	0.0009	<0.01

and systolic ($r = 0.32$, $P < 0.01$) and diastolic blood pressure ($r = 0.25$, $P < 0.01$) during a day and negatively with eGFR ($r = -0.295$, $P < 0.01$). No correlation between serum SDMA and ADMA was found. The factors that were found to have a significant correlation with serum SDMA and ADMA in the single regression analyses were used as explanatory variables to create the multiple regression models. To reduce the impact of multicollinearity we removed some correlated variables. Next we eliminated a few more nonsignificant (in the model) variables.

In multivariable analysis, serum ADMA was associated with serum uric acid, TG, and LDL-C independent of age, gender, and parameters of renal function (Table 2). On the other hand serum SDMA was associated with serum uric acid, serum creatinine, hs-CRP, and systolic blood pressure.

4. Discussion

In this cross-sectional study, we found that in adolescents with increased serum uric acid levels, serum SDMA is significantly elevated. We also confirmed positive correlation of serum SDMA with uric acid, creatinine, and hs-CRP and negative with eGFR. Additionally positive correlation between SDMA and systolic and diastolic blood pressure was found. It is interesting to note that we created multivariable linear regression model and found that serum uric acid, triglycerides, and LDL-cholesterol accounted for more than 37% of the variations in serum SDMA. Taken together, the results of this study demonstrate that serum SDMA is an independent determinant of renal function but also takes part in cardiovascular disease in pediatric patients with hyperuricemia.

Another important finding is that we have not found significant increase in serum ADMA between subjects with hyperuricemia and reference group; however, serum ADMA correlated positively with serum uric acid, BMI Z-score, triglycerides, total cholesterol, LDL-C, and hs-CRP. To our best knowledge this is the first clinical study on the association between the serum SDMA, ADMA, UA, and hs-CRP in adolescents with hyperuricemia.

In many previous reports it was shown that hyperuricemia is associated with endothelial dysfunction. The association of serum uric acid with endothelial dysfunction was reported by Zoccali et al. [14] in 217 individuals with mild untreated hypertension who underwent measurement

of endothelium-dependent brachial artery vasodilation. The authors reported that compared with participants whose serum UA was ≤ 3.5 mg/dL, endothelium-dependent vasodilation was 33% worse among those with serum UA levels of ≥ 5.5 mg/dL. Similar results were also reported by other authors [15, 16].

The effects of uric acid on the endothelium are subject of a debate. Uric acid has been shown to decrease NO production by endothelial cells in vitro [17]. Kang et al. [18] reported that fact in association with increased CRP expression. Furthermore, experimental studies showed that hyperuricemic rats developed endothelial dysfunction, but early L-arginine supplementation could prevent both the systemic and glomerular hypertension [19]. Taken together, these data suggest that higher levels of serum UA indicate the presence of endothelial dysfunction and might contribute to an increase in cardiovascular risk.

It is well established that atherosclerosis starts with endothelial injury [20]. In experimental studies it was shown that reduced NO bioavailability and oxidative stress contribute to endothelial dysfunction [21, 22].

Asymmetric dimethylarginine (ADMA) is an endogenous modulator of endothelial function and oxidative stress, and increased levels of this molecule have been reported in some metabolic disorders and cardiovascular diseases. Very little was found in the literature on role of ADMA in patients with features of metabolic syndrome. Palomo et al. [23] found that ADMA levels were significantly increased in the metabolic syndrome; however, the levels of ADMA were modestly correlated only with waist circumference but not with the other components of metabolic syndrome. In experimental study Korandji et al. [24] analyzed the relationship between dimethylarginine compounds and oxidative stress levels and cardiovascular function in fructose-hypertensive rats. The authors suggested that elevated levels of ADMA could in part be secondary to the early development of oxidative stress associated with development of hypertension. To our best knowledge no data concerning the relationship between the ADMA and uric acid in humans were published.

Several clinical studies have shown that ADMA levels are increased in hypertensive patients [25], atherosclerosis, and hypercholesterolemia [26]. Sladowska-Kozłowska et al. [27] analyzed oxidative stress in hypertensive children before and after 1 year of antihypertensive therapy. They found a positive correlation between ADMA and hs-CRP and TG/HDL ratio in patients with metabolic syndrome. Patients in whom ADMA concentrations decreased at followup had lower TG/HDL and LDL/HDL ratios when compared to patients with increase/stabilization of ADMA. Similarly Kanazawa et al. [28] found significantly positive association between ADMA and BMI, blood pressure, LDL, HDL and cholesterol independent of age. In obese women studied by Krzyzanowska et al. [29] ADMA correlated with high-sensitivity C-reactive protein at baseline and after weight loss, but no association with blood pressure or plasma lipids was observed.

In reviewing the literature, no information was found on the association between SDMA and metabolic syndrome. Very little is also known about the proinflammatory

properties of SDMA. Median serum SDMA levels in HU patients was significantly higher compared to the controls, similarly like hs-CRP, which was also confirmed in our previous study in similar population [30]. The results of this study showed that similarly ADMA and SDMA correlated positively with serum hs-CRP.

The link between UA and SDMA is not well known. Similarly we still do not know too much about the effect of SDMA on the L-arginine-NO pathway. Closs et al. [10] showed that SDMA had no effect on the inducible NOS extracted from macrophages. The results of Tojo et al. [31] experimental study suggested that SDMA might be a potent competitor of L-arginine transport and thereby have an indirect inhibitory effect of NO synthesis by limiting arginine availability to NOS. In human studies it was shown that SDMA was associated with inflammatory markers in patients with chronic kidney disease [32]. SDMA was independently associated with increased cardiovascular and total mortality in patients undergoing coronary angiography in studies by Meinitzer et al. [33] and Schulze et al. [34]. In contrast to studies presented above Zoccali et al. [35] did not confirm SDMA as a predictor of cardiovascular outcome in patients with chronic kidney disease. Both ADMA and SDMA were significantly elevated in patients with chronic renal failure; however, the increase was more pronounced for SDMA [36].

In conclusion, increased circulating methylarginines have been linked to some features of the metabolic syndrome; however, it is still difficult to answer the question about their role in this process. The results of the present study have demonstrated increased SDMA, but not ADMA levels in adolescents with hyperuricemia and their correlation with serum uric acid levels. However, at the moment it is difficult to answer the question if it is just coexistence of these factors or any mechanism linking uric acid and methylated arginines really exists.

Our study has limitations; it is a single-center, cross-sectional study, and confirmation in other cohorts is necessary to validate our findings. Second, the cohort was relatively small. Third, the study design did not allow to answer the question if SDMA is a new player or an innocent bystander in chronic inflammation in patients with hyperuricemia.

Abbreviations

ADMA: Asymmetric dimethylarginine
 BMI: Body mass index
 CVD: Cardiovascular disease
 hs-CRP: High sensitivity C-reactive protein
 FPG: Fasting plasma glucose
 NO: Nitric oxide
 NOS: Nitric oxide synthase
 NS: Not significant
 SDMA: Symmetric dimethylarginine
 HU: Hyperuricemic patients.

Conflict of Interests

The authors report no financial relationships or conflict of interests.

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