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ADMA, SDMA, L-ARGININE AND NITRIC OXIDE IN ALLERGIC PEDIATRIC BRONCHIAL ASTHMA

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Published data regarding asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine (L-ARG) and nitric oxide fraction in exhaled air (FeNO) in pediatric bronchial asthma are limited. Many question remain open about plasma concentration of these substances. The aim of this study is to evaluate ADMA, SDMA, L-ARG and FeNO concentration in allergic pediatric mild asthmatic patients in respect to healthy subjects. In this case-control study 60 children (50 asthmatics and 10 healthy) underwent a complete clinical visit, baseline respiratory function, allergy tests and biochemical analyses. The statistical significance of the different concentrations between the two groups were studied using one-way analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant. The mean plasma ADMA (0.58 *vs* 0.68 μmol/L), SDMA (0.40 *vs* 0.45 μmol/L) and L-ARG (52.2 *vs* 74.13 μmol/L) concentration were significantly lower (p<0.001) in the asthmatic patients in respect to healthy subjects (control group). The concentration of FeNO was significantly higher in the asthmatic subjects in respect to the control group (9.18 *vs* 4.2 μmol/L; p<0.001). Low plasma concentrations of ADMA, SDMA, L-ARG and high concentration of FeNO are associated with bronchial asthma and indicate an important role in airway disease through NO metabolism.

Nitric oxide (NO) is one of the most important mediators synthesized from L-arginine (L-ARG) by a family of NO syntheses (NOS), involved in the regulation of vascular tone, neurotransmission and mitochondrial respiration (1). The availability of NO in a given cell depends on many factors including expression and activity of several NOS (neuronal, inducible, and endothelial), abundance of NOS substrate, L-ARG, and its cofactor, tetrahydrobiopterin (THB) (2). NO production may also be regulated by endogenous NOS inhibitors, in particular asymmetric dimethylarginine (ADMA), synthesized during the methylation of protein arginine residues by protein arginine methyltransferases.

ADMA is a competitive inhibitor of NOS, decreases NO availability and is eliminated by renal excretion or metabolized by dimethylarginine dimethylaminohydrolases (DDAH) to citrulline and dimethylamine (3). Two other endogenous methylarginines are also synthesized by

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protein-arginine methyltransferases (PRMT): N-monomethyl-L-arginine (L-NMMA) and symmetric dimethylarginine (SDMA) (4). ADMA regulate NOS activity under physiological and pathological conditions, is metabolized by the endothelium and represent an important index of endothelial dysfunction (5). Published data regarding ADMA, SDMA, L-ARG and nitric oxide fraction in exhaled air (FeNO) in pediatric bronchial asthma are limited. The aim of this study is to evaluate ADMA, SDMA, L-ARG and FeNO concentration in mild asthmatic stable patients in respect to healthy subjects.

MATERIALS AND METHODS

Study population

Between March and July 2010 we enrolled 60 Caucasian subjects (50 asthmatics, 10 healthy; aged between 6 and 15 years), with at least a 2-year history of mild-persistent allergic bronchial asthma at Allergologic and Pneumological Service, (Department of Pediatric, University "G. D'Annunzio", Chieti, Italy). Medical and surgical history, physical condition, and medication were recorded. After inclusion, an EDTA or heparin blood sample was drawn from an indwelling arterial line for determination of ADMA, SDMA, L-ARG, and NO. Simultaneously, laboratory parameters indicating renal (creatinine, urea) and hepatic function [aspartate aminotransferase (AST), alanine aminotransferase (ALT)], complete haematocytometer exam, and baseline respiratory function test were determined. The diagnosis of allergic asthma was made by a pediatric respiratory physician and based on typical symptoms and laboratory tests, and according to ATS/ERS criteria (6). Allergic sensitization was evaluated by Skin Prick Test (SPT) and serum-specific IgE measurements for the most common respiratory allergens: Dust Mite (Dermatophagoides *Pteronyssinus*, and *Farinae*), Grass, *Parietaria*, *Artemisia Vulgaris*, Olive, Cypress, Lime, Stone, Elm, Plane, Cat and Dog dander, *Alternaria Alternata*, and *Aspergillus Fumigatus* (moulds).

Exclusion criteria for the study were: emergency treatment for an asthma exacerbation within the previous month; upper airway infections in the previous three weeks; hospitalization for asthma in the three months previous to enrolment; presence of autoimmune, hepatic or renal disorders, malabsorption, drug or alcohol-addiction. The study was approved by the Ethics Committee of the University of Chieti. Written informed consent was obtained from all parents and oral consent from all children.

Sample collection, storage and preparation

Blood samples were collected in polypropylene tubes containing 1 mM EDTA. Samples were stored in an ice box prior to centrifugation at 3000 g for 10 min at 4°C. 200 µl aliquots of plasma were transferred into Eppendorf tubes. Plasma samples were either used for extraction immediately or stored in the dark at -80°C until analysis was performed.

Biochemical analysis

The concentration of ADMA, SDMA and L-ARG were determined by high-performance liquid chromatography (HPLC) (7). In brief, solid-phase extraction on polymeric cation-exchange columns was performed after addition of monomethylarginine as the internal standard (IS). After derivatization with ortho-phtaldialdehyde reagent containing 3-mercaptopropionic acid, analytes were separated by isocratic reversed-phase HPLC with fluorescence detection (Figs. I and II).

Baseline functional respiratory test

Asthma was classified according to GINA guidelines at the beginning of the study (8). Subjects had to complete at least three forced vital capacity (FVC) to reach the ERS/ATS Guidelines (9).

Exhaled NO measurement

In all subjects FeNO value was determined with an on-line method using a single breath exhalation and a sensitive chemiluminescence assay (Ecomedics CLD 88), according to ATS-ERS procedures (10). Subjects made an inspiration of eNO-free air via a mouthpiece immediately followed by full exhalation at a constant rate (50mL/sec) for at least 5 seconds. The mean of three readings at the end of the expiration (plateau phase) was taken as the representative value for each measurements. Twelve ppb or more were considered elevated values, according to ATS-ERS criteria (11).

Statistical analysis

All values are expressed as means ± standard deviation (SD). Student's *t* test for paired data was used. Comparison between two groups was assessed using one-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison post-test. A p value <0.05 was required for statistical significance. Data were computed using the SPSS 7.0 statistical package.

RESULTS

Characteristics of subjects and lung function

Personal data and clinical details of subjects are summarized in Table I. We studied 60 subjects, 50

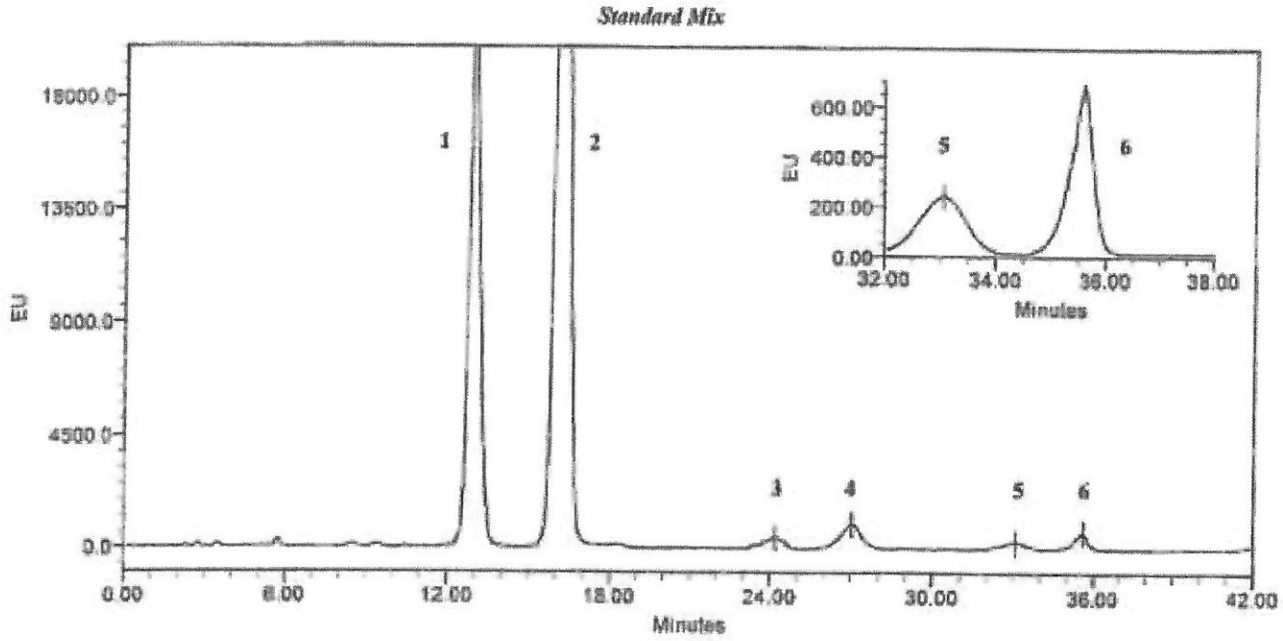

Fig. 1. *Chromatogram of combined standard containing: 50 μM citrulline, 120 μM arginine, 1.5 μM MMA (IS), 4 μM homoarginine, 1.2 μM ADMA, 1.7 μM SDMA. Peak identification: 1 citrulline; 2, arginine; 3, MMA (IS); 4, homoarginine; 5, ADMA; 6, SDMA.*

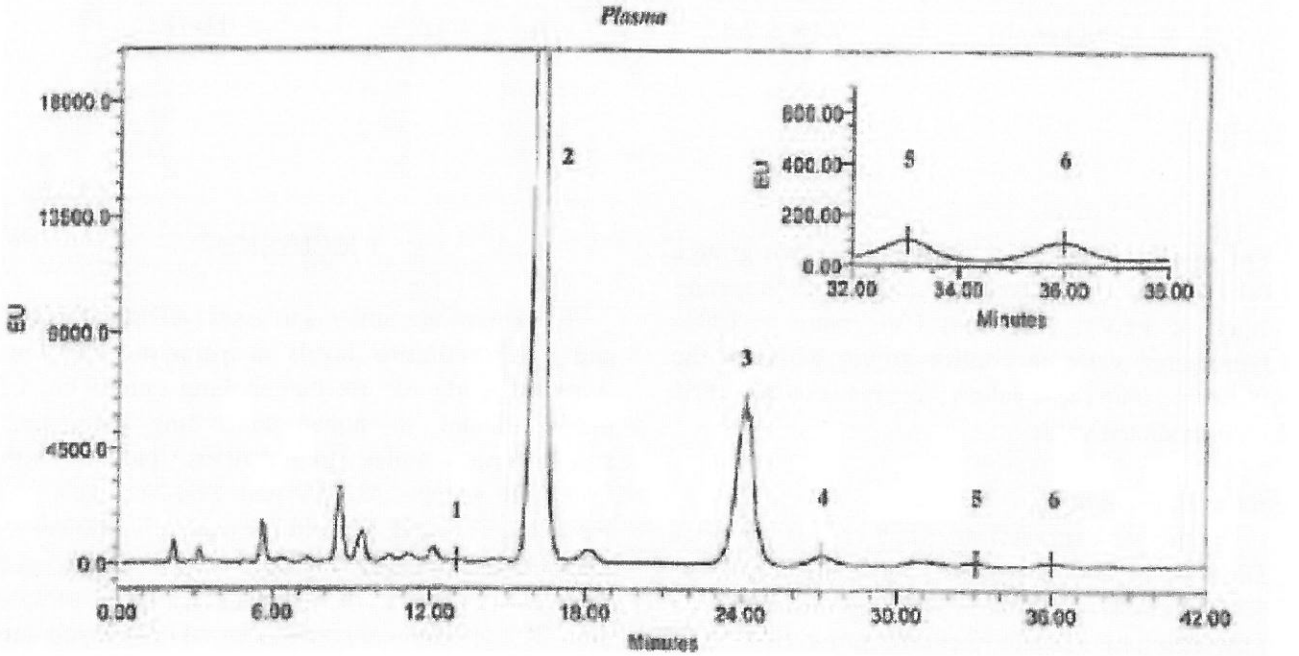

Fig. 2. *Plasma sample containing: citrulline (not quantified), 85 μM arginine, 25 μM MMA (IS), 1.6 μM homoarginine, 0,3 μM ADMA, 0,3 μM SDMA. Peak identification: 1 citrulline; 2, arginine; 3, MMA (IS); 4, homoarginine; 5, ADMA; 6, SDMA.*

asthmatics (mean age 11±2.5 yrs) and 10 healthy (mean age 11±2.1 yrs), 31 males (26 asthmatics) and 29 females (24 asthmatics). The two groups were similar for age, sex, weight, and height without significant differences. Regarding pulmonary parameters function (FEV1, PEF, and FVC) there

Table I. *Characteristic of subjects and spirometric values.*

	Asthmatics	Controls	p
No subjects	50 (26/24)	10 (5/5)	-
Sex (male/female)	26/24	5/5	ns
Age (years)	11 ± 2.5	11 ± 2.1	ns
Weight (kg)	40.7 ± 3.78	41.1 ± 4.14	ns
Height (cm)	142 ± 7.5	141 ± 6.5	ns
Spirometric values			
FEV1 (% predicted)	85 ± 12	100 ± 10.2	< 0.01
FVC (% predicted)	86 ± 11	99 ± 11.7	< 0.01
PEF (% predicted)	88 ± 10	101 ± 10.5	< 0.01

Table II. *Biochemical analyses.*

	Asthmatics	Controls	p
ADMA (μmol/L)	0.58 ± 0.05	0.68 ± 0.06	< 0.001
SDMA (μmol/L)	0.40 ± 0.03	0.45 ± 0.03	< 0.001
L-ARG (μmol/L)	52.2 ± 10.5	74.13 ± 11.2	< 0.001
FeNO (ppb)	9.18 ± 2.12	4.2 ± 1.12	< 0.001

were significant differences among the two groups (asthmatic *vs* control groups). Lung function testing values of FEV1, PEF, and FVC were > 100% of predicted vales in control group, while in the asthmatic group these values were between 80-100% of the predicted value.

Biochemical analyses

The mean plasma ADMA (0.58 *vs* 0.68 μmol/L), SDMA (0.40 *vs* 0.45 μmol/L) and L-ARG (52.2 *vs* 74.13 μmol/L) concentration were significantly lower in the asthmatic respect to control group ($p<0.001$). The concentration of FeNO was significantly higher in the asthmatic subjects in respect to the control group (9.18 *vs* 4.2 ppb; $p<0.001$) (see Table II). There was no difference in laboratory parameters indicating renal (creatinine, urea) and hepatic (AST, ALT) functions, and complete hematologic examination (hemoglobin, red and white cells, hematocrit) between the two groups.

DISCUSSION

The aim of this study is to assess ADMA, SDMA and L-ARG plasma levels in asthmatic children. A recent study reports that in lung epithelium of murine model, in human adult lung specimens, and sputum samples from pediatric patients with bronchial asthma ADMA and SDMA levels are increased 1.7- and 1.8-fold, respectively. However, these differences did not reach statistical significance for ADMA because of the relatively small sample size. The authors concluded that ADMA levels are increased in asthma and contribute to NOS-related pathophysiology (12). In our study ADMA, SDMA and L-ARG plasma levels of asthmatic children significantly decreased in respect to the control group, and FeNO level increases. ADMA and SDMA plasma values were decreased to improve NOS activity and increase NO level in systemic pathways of L-ARG. Little is known about ADMA metabolism

in the pathogenesis of bronchial asthma. ADMA and SDMA are synthesized during the methylation of protein arginine residues by S-adenosylmethionine: protein arginine methyltransferases (protein methylases, PMRT), which exist in multiple isoforms encoded by separate genes (13). We suggest that the difference of ADMA and SDMA found in sputum samples and lung epithelium derives from compensatory mechanism to eliminate local ADMA and SDMA resulting from epithelial injury and as another physiological bronchial excretion way (14).

Arginine (ARG) is one of the 20 amino acids (AA) found in proteins and synthesized by human cells. However, ARG is also the substrate for a series of reactions leading to the synthesis of other AA and is an obligatory substrate for two enzymes with diverging actions, arginases and NOS, giving origin to urea and NO, respectively. Intracellular L-ARG levels are regulated by at least three distinct mechanisms: (a) cellular uptake by cationic amino acid (CAT) transporters, (b) metabolism NOS and arginases, and (c) recycling from L-citrulline. Changes in L-ARG homeostasis may contribute to asthma disease by increased production of NO, a potent vasodilator when produced by eNOS. Airway inflammation is associated with an enhanced expression of iNOS (15, 16).

Measurement of FeNO is a very useful non-invasive method in the treatment monitoring of asthma (11, 16). A large body of scientific literature suggests that high NO plasma levels correlate to some pulmonary diseases, such as asthma, and corticosteroid treatment decreases NO levels in exacerbation asthma cases (11, 17). Published studies have shown a significant correlation between FeNO and respiratory symptoms, bronchial hyperresponsiveness (BHR), and airways inflammation (18-21).

Recent studies revealed that FeNO is a potentially useful measure to evaluate the role of airways inflammation in asthma, as it represents the forerunner of an important event in asthma: the remodeling of bronchial airways (22). Increased FeNO value and decreased L-ARG plasma level, indeed confirm that L-ARG is metabolized by NOS to form NO and produce Ng-hydroxy-L-ARG, which inhibits the arginase pathway (23-25).

Our findings reveal that L-ARG, ADMA, SDMA and NO in exhaled air are correlated with airway inflammatory diseases. Thus, further studies are desirable to examine the role of L-ARG, ADMA, SDMA plasma levels and exhaled NO.

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