Free Radical Research, February 2011; 45(2): 147–155

Association of serum arginase I with oxidative stress in a healthy population

KEIKI OGINO[1], NORIKO TAKAHASHI[1], TOMOKO TAKIGAWA[1], YASUSHI OBASE[2] & DA-HONG WANG[1]

[1]*Department of Public Health, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan, and* [2]*Division of Respiratory Diseases, Department of Medicine, Kawasaki Medical School, Okayama, Japan*

(Received date: 8 July 2010; In revised form date: 28 August 2010)

Abstract

The association of serum arginase I with oxidative stress was evaluated cross-sectionally in a healthy population. The mean levels of serum arginase I in healthy people (n = 278) were 32.6 ± 22.3 ng/ml. Significant correlations of arginase I were observed with age, WBC, RBC, alanine aminotransferase (ALT), high-sensitivity C-reactive protein (hs-CRP), uric acid, body mass index (BMI) and urinary 8-isoprostane. Multiple regression analysis showed significant associations of arginase I with WBC, RBC, urinary 8-hydroxydeoxyguanosine (8-OHdG), age, HbA1c and urinary 8-isoprostane. In the associations of arginase I with 8-OHdG, 8-isoprostane and HbA1c, confounding factors and lifestyle factors such as sex, old age, smoking and alcohol consumption were involved. It was concluded that serum arginase I was associated with oxidative stress and HbA1c in addition to age, WBC and RBC in healthy Japanese people and may become a new biomarker for early prediction of diabetes mellitus and other oxidative stress-related diseases.

Keywords: *Arginase I, oxidative stress, population study, healthy people*

Introduction

Two distinct isoforms for arginase, arginase I and II, which differ in sub-cellular localization, are known. Arginase I is predominantly expressed in cytosol of hepatic cells as a key enzyme for the urea cycle and arginase II is expressed in mitochondria of extra-hepatic cells encoded by a different gene [1]. Both isozymes are constitutively expressed in cells and tissues and regulate nitric oxide (NO) generation from nitric oxide synthase (NOS) by competition of a common enzyme substrate, L-arginine, which is metabolized to L-citrulline by NOS and to L-ornithine by arginase [2–4]. Therefore, the induction of arginase in the lung including the airway epithelium and alveolar macrophages has been focused on as pathophysiological evidence that the consumption of L-arginine by arginase may lead the depletion of NO to enlarge bronchial smooth muscle associated with airway hyper-responsiveness [5–7]. However, there is an important difference in the expression of arginase I between rodents and humans. In haematopoietic cells (erythrocytes and granulocytes), arginase I is not expressed in rodents but in humans [8].

Serum arginase levels were evaluated in various diseases by activity assay and the ELISA method. In asthmatic patients, no agreement was established in terms of serum activity for arginase [9–12]. Patients with sickle cell disease suffered from asthma and pulmonary hypertension due to increased serum arginase levels by haemolysis [13,14]. In colorectal cancer, serum ELISA levels of arginase I increased [15]. In renal cell carcinoma and pancreatic cancer, an increase in myeloid-derived suppressor cells (MDSC) was found to be associated with T-cell dysfunction by the depletion of L-arginine due to a high level of arginase I released from MDSC [16].

Correspondence: Keiki Ogino, Department of Public Health, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan. Fax: +81 86 2260715. Email: kogino@md.okayama-u.ac.jp

ISSN 1071-5762 print/ISSN 1029-2470 online © 2011 Informa UK, Ltd.
DOI: 10.3109/10715762.2010.520318

However, little is known about arginase I in healthy populations [17].

Serum levels of arginase may be influenced by pathophysiological changes of the tissue such as inflammation and carcinogenesis. Induction of arginase was reported in various rodent cells by many stimulants of Th2 cytokines such as IL-4 and IL-13, growth factors, endotoxin, cAMP-elevating agents, oxygen tension and reactive oxygen species (ROS) [18,19]. However, in human cells, there is little evidence for the inducer of arginase I such as nicotine in cigarette smokers [20] and pregnancy-specific glycoprotein 1a [21]. Although induction of arginase I by IL-4 or IL-13 was found to be mediated by a nuclear transfer factor, STAT 6, in the lung of asthmatic mice model [22], induction of arginase I was not demonstrated by IL-13 or IL-4 in human cells [8].

In terms of oxidative stress, several biomarkers are known. 8-Hydroxy deoxyguanosine (OHdG), a product of the oxidatively modified DNA base guanine, is the most representative product that may reflect oxidative damage induced by ROS [23]. In healthy populations, urinary 8-OHdG is associated with urinary metals such as arsenic or chromium, age, BMI and fasting insulin [24,25]. 8-Isoprostane, a group of bioactive prostaglandin F2-like compounds generated by oxidatively catalysed reactions of arachidonic acid, are considered as reliable markers of lipid peroxidation *in vivo* [26]. In healthy populations, urinary 8-isoprostane is associated with sex and alcohol consumption [25].

Therefore, in this study, we demonstrate the interaction of arginase I with several clinical parameters including oxidative stress biomarkers in a healthy population to identify ROS as inducers of arginase I in humans.

Materials and methods

Study design

From 847 individuals who undertook a worksite lifestyle study in Okayama city, 300 healthy individuals were selected by stratified sampling in sex between male and female and in age between under 45 years old and over 45 years old; then 257 individuals who had no previous history of cancer, stroke, diabetes, ischemic heart disease, liver disease, asthma, other allergic diseases (allergic rhinitis, atopic dermatitis, pollinosis) and the serum of which did not appear to be haemolytic were finally selected. The ethics committee of Okayama University approved the study and all subjects gave informed consent.

Measurement of parameters for whole blood and serum

Blood samples were collected after overnight fasting. Whole blood and separated serum were measured for haematological parameters (RBC and WBC) and serum alanine aminotransferase (ALT) because arginase I is constitutively expressed in granulocytes, red blood cells and liver cells. Low density lipoprotein (LDL-c), uric acid and high-sensitivity C-reactive protein (hs-CRP) were measured to evaluate the correlation of arginase I with atherosclerosis. Haemoglobin A1c (HbA1c) was measured for the evaluation of diabetes mellitus. Serum arginase I levels were determined using ELISA kits of Bethyl Laboratories (Montgomery, TX, The Netherlands) and Hycult Biotechnology b.v. UDEN, and then other clinical examinations involving haematological parameters were performed using automated XE-2100 (Sysmex, Kobe, Japan) and H7700 (Hitachi High-Technologies, Kobe, Japan). Serum hs-CRP was measured by a highly sensitive CRP assay (Behring Latex-Enhanced using the Behring Nephelometer BN-100; Behring Diagnostics, Westwood, MA). NOx (NO_2^- + NO_3^-) levels in the serum were determined with a NO analyser (model-280i NOA with the Purge Vessel; Sievers, Boulder, CO) to evaluate nitric oxide (NO) generation [25]. Serum was treated with nitrate reductase (Sigma-Aldrich, St. Louis, MO, Tokyo) to convert nitrate to nitrite for 30 min at room temperature. Nitrite was further reduced to NO in the Purge Vessel containing the reducing agent potassium iodide in acetic acid and NO was subsequently detected by the ozone-chemiluminescence method. Information on lifestyle factors including cigarette smoking, past history and present steroid medication was obtained using self-reported questionnaires or clinical records.

Analysis of urinary oxidative stress biomarkers

Urinary 8-isoprostane and 8-OHdG were determined using a commercially available competitive enzyme immunoassay (EIA) kit (Cayman Chemical Company, Ann Arbor, MI) and an ELISA kit from the Japan Institute for the Control of Aging (Fukuroi, Shizuoka, Japan), respectively. Spot urine samples stored at –80°C before analysis were used because spot urine for 8-isoprostane did not exhibit significant variation from levels measured in 24-h urine samples in the same healthy individuals by radioimmunoassay [27] and the correlation coefficient of 8-OHdG by ELISA between spot and 24-h urine samples was 0.87 [28]. The intra-assay and inter-assay CV were 5.4% and 11.0% for 8-isoprostane and 5.2% and 8.1% for 8-OHdG, respectively.

Urinary hydrogen peroxide (H_2O_2) was measured by the ferrous ion oxidation xylenol orange version-2 (FOX-2) method [29]. FOX-2 reagents contained 100 mM xylenol orange, 250 mM ammonium ferrous sulphate, 100 mM sorbitol and 25 mM H_2SO_4. Fifty microlitres of test sample was added to 950 µl FOX-1

reagent, voltexed and incubated at room temperature for 30 min and then absorbance at 560 nm was measured. H_2O_2 (0–50 μM) was added in the test sample as a standard and 2200 U/ml catalase was added as a blank control. The intra-assay and inter-assay CV for the measurement of urinary H_2O_2 by FOX-1 were 3.37% and 4.98%, respectively [30]. Values for 8-isoprostane, 8-OHdG and H_2O_2 were normalized per milligram of creatinine (Cre) measured in urine (Creatinine Test Kit, R&D Systems, Minneapolis, MN).

Statistical analysis

Statistical analyses were performed by GraphPad Prism 5.0c for Mac (GraphPad Software, Inc., San Diego, CA) and PASW Statistics 18 for Mac. Results are expressed as means ± SD. Gaussian distribution of values was analysed by Shapiro-Wilk normality test. Unpaired *t*-test and ANOVA were used to examine the differences in serum levels of arginase I among the variables such as sex, age, smoking and alcohol consumption. Pearson's correlation was used to examine the relationship between serum arginase I and clinical parameters. A stepwise method for multiple regression analysis was performed to test the relationship between arginase I levels and the variables that had significant correlation as tested using Pearson's correlation and the variables that are presumably confounding for arginase I such as age and sex. A p-value < 0.05 was considered statistically significant.

Results

Characteristics of subjects by arginase I

The characteristics of the subjects by serum arginase I in this study are presented in Table I. Their average age was 43.8 years. Smokers accounted for 41.4% and the alcohol consumption rate was 68.7%. The serum concentration of arginase I in healthy controls (n = 278) was 32.6 ± 22.3 ng/ml. A difference in arginase I was not observed in terms of sex, smoking and alcohol consumption. However, concerning age, healthy persons under 45 years old showed significantly high levels of serum arginase I compared with those over 45 years old (Table I). Urinary 8-OHdG showed significantly high levels in males and persons over 45 years old compared with the levels in females and persons under 45 years old. Urinary 8-isoprostane showed significantly high levels in smokers compared with the levels in non-smokers.

Pearson's correlation analysis between arginase I and clinical examination variables including oxidative stress biomarkers

The results of Pearson's correlation analysis between oxidative stress biomarkers and health assessment data are presented in Table II. Serum levels of arginase I were significantly positively correlated with WBC, RBC, ALT, hs-CRP, urinary 8-isoprostane, uric acid and BMI and negatively correlated with age in all healthy populations (Figure 1). Healthy persons were stratified by sex (male or female), age (under 45 years old or over 45 years old), smoking status (smoker or non-smoker) and alcohol consumption (alcohol drinker or non-drinker). A significant positive correlation of arginase I with WBC was observed in almost all population groups. RBC also showed positive correlations with arginase I in population groups of males and persons over 45 years old. As for age, a significant negative correlation with arginase I was observed in females, smokers and those who do not consume alcohol. ALT and hs-CRP showed almost the same significant correlations with males, persons under 45 years old and smokers. However, concerning alcohol intake, ALT showed a significant correlation, but CRP did not show a significant correlation. Among three urinary oxidative biomarkers, urinary 8-OHdG showed significantly positive correlations with arginase I in females and persons under 45 years old, but not in the entire population; urinary 8-isoprostane showed significant correlations with arginase I in the entire population, smokers and those who consume alcohol. Uric acid, a serum antioxidant, was positively correlated with arginase I in the entire population, males, persons under 45 years old and smokers. HbA1c, a biomarker of diabetes mellitus, showed significantly positive correlations with females, persons over 45 years old and non-smokers. BMI, a biomarker of obesity, was significantly correlated with arginase I in the entire population, males, persons under 45 or over 45 years old, smokers, and those who consume alcohol.

Multiple regression analysis for arginase I with clinical examination variables including oxidative stress biomarkers

Multiple regression analysis results by a stepwise method are shown in Table III. Significant associations of arginase I with WBC, RBC, urinary 8-OHdG, age, HbA1c and urinary 8-isoprostane were observed in the entire population. That is to say, WBC, RBC, urinary 8-OHdG, age, HbA1c and urinary 8-isoprostane were important influential factors of serum arginase I, independent of sex, hs-CRP, LDL-C, uric acid and BMI. In older persons, serum arginase I will decrease. If urinary 8-OHdG, HbA1c and urinary 8-isoprostane increase, serum arginase I will increase. In this model, 16.4% of the variation of arginase I was explained. By stratification of the entire population into sex (male or female), age (under 45 or over 45), smoking status (smoker or non-smoker) and alcohol consumption (alcohol drinker or non-drinker), a negative association of arginase I with age was shown to be dependent

Table I. Characteristics of subjects by several clinical parameters including oxidative stress markers.

Variable	All (n = 278)	Male (n = 142; 51.0%)	Female (n = 136; 49.0%)	P	Age < 45 (n = 134; 48.2%)	Age ≥ 45 (n = 144; 51.8%)	P
Age	43.5 ± 11.2	43.6 ± 11.3	43.4 ± 11.2	0.911	–	–	
Age < 45/ ≥ 45	121/136	68/74	66/70	–	–	–	–
Male/Female	134/123	–	–	–	68/66	74/70	–
Arginase I (ng/ml)	32.6 ± 22.3	32.5 ± 22.4	32.6 ± 22.2	0.957	36.2 ± 23.3	29.2 ± 20.8	**0.009**
WBC (/mL)	5917 ± 1606	5780 ± 1619	6059 ± 1587	0.149	5957 ± 1619	5878 ± 1600	0.683
RBC (/mL)	469 ± 46	450 ± 33	488 ± 50	**<0.001**	478 ± 42	460 ± 48	**0.001**
ALT (IU/L)	22.6 ± 18.6	18.3 ± 14.4	27.1 ± 21.3	**<0.001**	23.2 ± 23.5	22.0 ± 12.5	0.582
hs-CRP (mg/dL)	0.077 ± 0.111	0.074 ± 0.116	0.080 ± 0.106	0.634	0.071 ± 0.108	0.082 ± 0.114	0.427
NOx (μM)	28.7 ± 16.2	27.5 ± 15.5	30 ± 16.8	0.202	26.4 ± 14.1	30.8 ± 17.7	**0.022**
8-OHdG (ng/mg Cre)	10.5 ± 4.6	11.5 ± 5.2	9.4 ± 3.4	**<0.001**	9.3 ± 3.9	11.6 ± 4.8	**<0.001**
8-Isoprostane (pg/mg Cre)	763.3 ± 619.5	701.0 ± 692.8	828.4 ± 527.4	0.087	793.6 ± 533.0	735.2 ± 691.1	0.433
H_2O_2 (μM/g Cre)	5.9 ± 9.7	6.5 ± 12.0	5.4 ± 6.4	0.322	4.8 ± 6.4	7.0 ± 11.9	0.058
LDL-C (mg/dL)	129.9 ± 37.1	129.5 ± 41.3	130.4 ± 32.4	0.842	117.8 ± 32.2	141.2 ± 38.0	**<0.001**
Uric acid (mg/dL)	5.3 ± 1.4	4.5 ± 1.3	6.1 ± 1.2	**<0.001**	5.2 ± 1.4	5.4 ± 1.4	0.199
HbA1c (%)	5.0 ± 0.6	5.0 ± 0.5	5.0 ± 0.7	0.498	4.8 ± 0.3	5.2 ± 0.7	**<0.001**
BMI (kg/m^2)	23.0 ± 4.1	22.4 ± 4.2	23.8 ± 3.8	**0.004**	22.6 ± 4.3	23.3 ± 3.8	0.160

Variable	Smoking (+) (n =115; 41.4%)	Smoking (–) (n = 163; 58.6%)	P	Alcohol (+) (n = 191; 68.7%)	Alcohol (–) (n = 87; 31.3%)	P
Age	42.5 ± 10.8	44.3 ± 11.5	0.195	43.6 ± 11.4	43.4 ± 10.8	0.872
Age < 45/≥ 45	62/53	71/92	–	91/100	41/46	–
Male/Female	44/71	96/67	–	85/106	57/30	–
Arginase I (ng/ml)	33.6 ± 21.1	31.8 ± 23.1	0.506	32.2 ± 20.6	33.7 ± 25.7	0.600
WBC (/mL)	6245 ± 1665	5685 ± 1527	**0.004**	6005 ± 1630	5723 ± 1544	0.176
RBC (/mL)	473 ± 39	466 ± 51	0.243	470 ± 49	466 ± 40	0.536
ALT (IU/L)	20.8 ± 16.1	23.9 ± 20.1	0.171	22.7 ± 17.3	22.5 ± 21.3	0.943
hs-CRP (mg/dL)	0.077 ± 0.108	0.076 ± 0.113	0.944	0.082 ± 0.119	0.066 ± 0.092	0.273
NOx (μM)	27.4 ± 15.6	29.6 ± 16.5	0.276	29.8 ± 17.4	26.4 ± 12.9	0.102
8-OHdG (ng/mg Cre)	9.9 ± 4.1	10.8 ± 4.8	0.103	10.5 ± 4.3	10.3 ± 5.0	0.686
8-Isoprostane (pg/mg Cre)	892.4 ± 524	672.2 ± 665.7	**0.003**	792.3 ± 645.7	699.7 ± 556.1	0.249
H_2O_2(μM/g Cre)	4.3 ± 5.8	7.1 ± 11.5	**0.018**	6.3 ± 10.6	5.4 ± 7.3	0.488
LDL-C (mg/dL)	130.9 ± 39.3	129.2 ± 35.7	0.704	126.9 ± 36.6	136.5 ± 37.7	**0.046**
Uric acid (mg/dL)	5.3 ± 1.4	5.3 ± 1.5	0.901	5.5 ± 1.5	4.8 ± 1.2	**< 0.001**
HbA1c (%)	5.0 ± 0.4	5.1 ± 0.7	0.140	5.0 ± 0.5	5.1 ± 0.7	0.386
BMI (kg/m^2)	22.5 ± 3.6	23.4 ± 4.3	0.054	23.3 ± 4.2	22.5 ± 3.8	0.150

P value of difference in sex , age, smoking and alcohol inatke was analyzed by unpaired t test. AST indicates aspartate aminotransferase; hs-CRP, high-sensitivity C-reactive protein; 8-OHdG, 8-hydroxy deoxyguanosine; LDL-C, low-density lipoprotein; BMI, body mass index.

Table II. Pearson's correlation of arginase I with several clinical parameters including oxidative stress markers.

Variable	All (278)		Male (142)		Female (136)		Age < 45 (134)		Age ≥ 45 (144)		Smoking (+) (115)		Smoking (−) (163)		Alcohol (+)(191)		Alcohol (−) (87)	
	r	P	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Age	**−0.128**	**0.032**	−0.051	0.547	**−0.211**	**0.014**	−0.008	0.924	0.025	0.763	**−0.197**	**0.034**	−0.082	0.296	−0.131	0.071	**−0.266**	**0.013**
WBC	**0.302**	**<0.0001**	**0.338**	**<0.0001**	**0.265**	**0.002**	**0.402**	**<0.0001**	**0.197**	**0.018**	**0.253**	**0.006**	**0.334**	**<0.0001**	**0.274**	**<0.001**	**0.279**	**0.009**
RBC	**0.218**	**<0.001**	0.129	0.125	**0.321**	**<0.001**	**0.278**	**0.001**	0.119	0.157	**0.250**	**0.007**	**0.199**	**0.011**	**0.191**	**0.008**	**0.306**	**0.004**
ALT	**0.153**	**0.011**	**0.186**	**0.027**	0.142	0.100	**0.171**	**0.048**	0.121	0.147	0.074	0.431	**0.200**	**0.010**	0.076	0.298	**0.226**	**0.035**
hs-CRP	**0.125**	**0.037**	**0.203**	**0.016**	0.035	0.684	**0.204**	**0.018**	0.066	0.429	0.075	0.427	**0.156**	**0.047**	0.102	0.160	0.184	0.088
NOx	0.014	0.822	0.124	0.143	−0.094	0.275	0.071	0.417	0.007	0.930	−0.072	0.443	0.070	0.376	−0.057	0.430	0.152	0.159
8-OHdG	0.101	0.093	0.060	0.477	**0.181**	**0.035**	**0.191**	**0.027**	0.113	0.178	0.154	0.100	0.079	0.318	0.086	0.239	0.078	0.475
8-Isoprostane	**0.121**	**0.044**	0.156	0.064	0.075	0.386	0.091	0.293	0.137	0.101	**0.188**	**0.045**	0.080	0.313	**0.159**	**0.028**	0.034	0.755
H_2O_2	−0.090	0.132	−0.108	0.203	−0.068	0.431	−0.011	0.898	−0.117	0.163	−0.033	0.730	−0.107	0.176	−0.109	0.134	-0.066	0.545
LDL-C	0.004	0.946	0.023	0.783	−0.022	0.801	0.122	0.159	0.000	0.999	−0.029	0.758	0.026	0.742	−0.027	0.706	-0.060	0.583
Uric acid	**0.133**	**0.026**	**0.279**	**0.001**	0.023	0.794	**0.179**	**0.039**	0.116	0.167	0.013	0.889	**0.206**	**0.008**	0.107	0.139	0.161	0.136
HbA1c	0.112	0.062	0.008	0.924	**0.198**	**0.021**	0.122	0.161	**0.212**	**0.011**	−0.085	0.364	**0.202**	**0.010**	−0.015	0.838	0.008	0.942
BMI	**0.187**	**0.002**	**0.296**	**<0.001**	0.065	0.454	**0.181**	**0.037**	**0.211**	**0.011**	0.095	0.313	**0.247**	**0.001**	**0.162**	**0.025**	0.197	0.067

on females and smokers. An association of arginase I with urinary 8-OHdG was dependent on females, those under 45 years old and smokers and then an association of arginase I with urinary 8-isoprostane was shown to be dependent on smokers and those who consume alcohol. Moreover, an association of arginase I with HbA1c was dependent on females, those over 45 years old and non-smokers.

Discussion

An ELISA system for arginase I was developed in the field of liver injury because of the high level of arginase I in the liver. Moreover, in sickle cell disease (SCD), serum arginase I levels are crucial for pulmonary hypertension and asthma owing to haemolysis-derived elevation of arginase I. Serum arginase I levels in normal controls were reported to be 51.0 ± 3.3 ng/ml [31] and 20 ± 6 ng/ml [32]. Our result, 31.7 ± 22.2 ng/ml, is similar to previous results. However, the characteristics of serum arginase I by ELISA in healthy populations were not known. One report suggested a positive correlation of arginase I with age in females (n = 60) [31]. In this study, negative correlations of arginase I with age were observed not only in the entire healthy population but also in females (n = 136). The reason for this discrepancy is not clear.

Various inducers of arginase I were demonstrated in murine mononuclear cells [18,19]. Among these inducers, much attention should be paid to ROS because arginase I was constitutively expressed in neutrophils [8] and activated neutrophils can generate ROS together with degranulation. Although there is no evidence demonstrating the induction of arginase I in human cell lines, H_2O_2 or hydroxyl radical was involved in the induction of arginase I in rodents macrophages [33] and porcine vascular endothelial cells [34]. In the mechanisms for the induction of arginase I by H_2O_2 or hydroxyl radical, nuclear transcription factors such as NFκ-B and AP-1, known to be activated by ROS, may be involved [35,36]. Therefore, in human neutrophils, it is speculated that activation of neutrophils or natural apoptosis-related neutrophil death [37] accompanied by oxy-radical generation and release of arginase I from neutrophils may occur. In this study, association of serum arginase I with urinary levels of 8-OHdG was observed in females, persons under 45 years old and smokers and that with 8-isoprostane was observed in smokers and those who consume alcohol. Previously, we demonstrated significant correlations of urinary 8-isoprostane with smoking status and alcohol consumption in a healthy population [24,25]. The generation of 8-isoprostane and 8-OHdG is closely associated with oxidative stress [23]. These findings suggest that the release of arginase I from neutrophils may be regulated by oxidative stress, although there

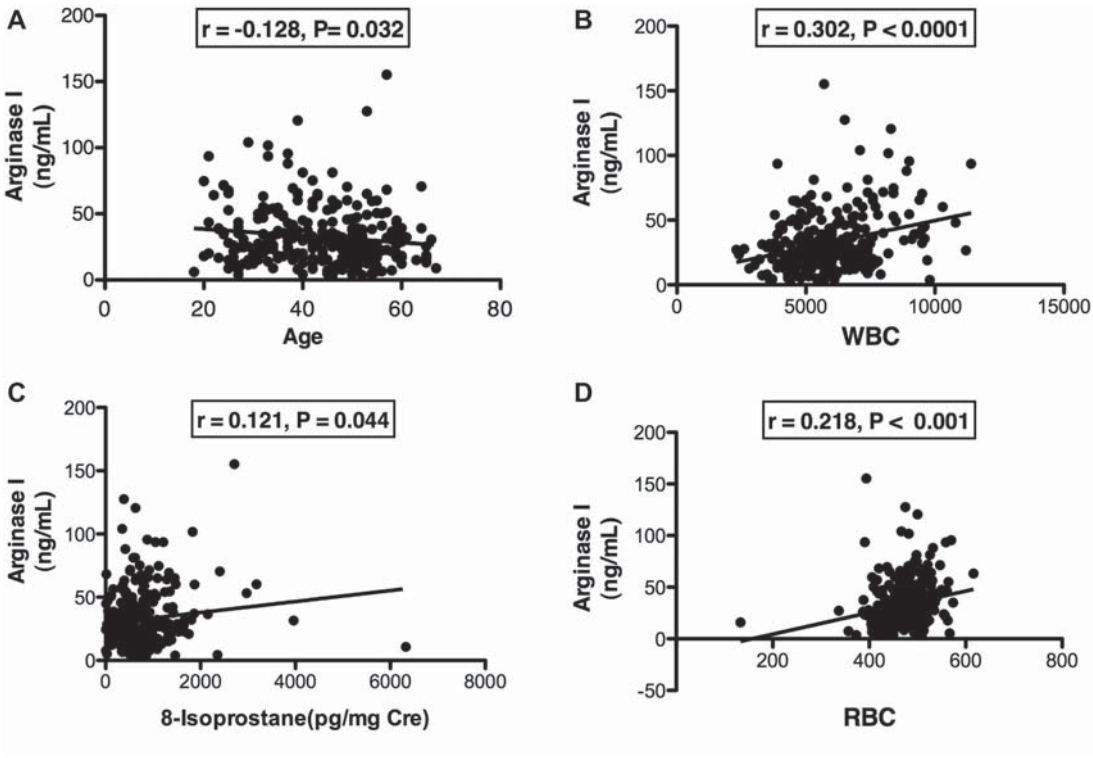

Figure 1. Pearson's correlation. Serum arginase I was significantly correlated with age (A), WBC (B), 8-isoprostane (C) and RBC (D).

is no direct evidence that oxyradicals induce arginase I in neutrophils or monocytes in humans.

An association of serum arginase I with HbA1c was observed. HbA1c is a useful marker for the diagnosis of diabetes mellitus. High HbA1c means a long-term elevation of serum glucose. Chemical changes in haemoglobin occur in high serum glucose by Amadori rearrangement. During this reaction, superoxide

Table III. Multiple regression analysis for arginase I by stepwise method.

Group	Explanatory variable	β	*p*	Adjusted R^2	Group	Explanatory variable	β	*p*	Adjusted R^2
				0.164	Age ≥ 45				0.066
All	WBC	0.250	<0.001			HbA1c	0.201	0.014	
	RBC	0.147	0.012			WBC	0.185	0.024	
	8-OHdG	0.178	0.002						
	Age	–0.185	0.003		Smoking (+)				0.164
	HbA1c	0.159	0.007			WBC	0.229	0.010	
	8-Isoprostane	0.126	0.025			Age	–0.300	0.001	
						8-OHdG	2.635	0.010	
Male	WBC	0.288	<0.001	0.144		8-Isoprostane	2.390	0.019	
	Uric acid	0.212	0.009		Smoking (−)	WBC	0.323	<0.001	0.134
Female	WBC	0.217	0.005	0.253		HbA1c	0.182	0.014	
	RBC	0.210	0.010		Alcohol (+)				0.086
	HbA1c	0.285	0.005			WBC	0.265	<0.001	
	Age	–0.277	0.001			8-Isoprostane	0.141	0.043	
	8-OHdG	0.222	0.004		Alcohol (−)	RBC	0.319	<0.001	0.201
Age < 45				0.215		WBC	0.292	0.001	
	WBC	0.344	<0.001			Sex	–0.253	0.023	
	RBC	0.231	0.004						
	8-OHdG	0.159	0.042						

β indicates standardized partial regression coefficient.
Variables included in the model for all: sex, ALT, NOx, H_2O_2, LDL-C, uric acid, BMI; for males: age, RBC, ALT, NOx, 8-OHdG, 8-isoprostane, H_2O_2, LDL-C, HbA1c, BMI; for females: ALT, NOx, 8-isoprostane, H_2O_2, LDL-C, uric acid, BMI; for age < 45: sex, ALT, NOx, 8-isoprostane, H_2O_2, LDL-C, uric acid, HbA1c, BMI; for age ≥ 45: sex, RBC, ALT, NOx, 8-OHdG, 8-isoprostane, H_2O_2, LDL-C, uric acid, BMI; for smoking (+): sex, RBC, ALT, NOx, H_2O_2, LDL-C, uric acid, HbA1c, BMI; for smoking (-): age, sex, RBC, ALT, NOx, 8-OHdG, 8-isoprostane, H_2O_2, LDL-C, BMI; for alcohol (+): age, sex, RBC, ALT, NOx, 8-OHdG, H_2O_2, LDL-C, uric acid, HbA1c, BMI; for alcohol (−): age, ALT, NOx, 8-OHdG, 8-isoprostane, H_2O_2, LDL-C, uric acid, HbA1c, BMI. 8-OHdG indicates 8-hydroxyl deoxyguanosine.

radicals are simultaneously generated. Moreover, ROS are produced in various tissues and cells under diabetic conditions [38,39] from the electron transport chain in mitochondria [40] and membrane-bound NADPH oxidase [41], with the exception of the non-enzymatic glycosylation reaction [42]. High arginase activities were observed in the serum of diabetes mellitus patients [43]. Therefore, the association of serum arginase I with HbA1c may contribute to oxidative stress.

Our study found that uric acid, BMI, ALT as well as hs-CRP had significant correlations with arginase I by Pearson's correlation analysis. However, the associations of arginase I with BMI, ALT and hs-CRP were not found by multiple linear regression analysis. Therefore, correlations of arginase I with BMI, ALT and hs-CRP may be confounded via other variables such as RBC or WBC which had high correlations with arginase I, BMI, ALT and hs-CRP. Moreover, arginase I is constitutively expressed in hepatocytes in addition to neutrophils and red blood cells in humans. In patients with acute hepatic injury, transiently increased levels of serum arginase I returned quickly to normal levels compared with ALT and AST [44]. Therefore, although there is no data showing the contribution of hepatic release to serum levels of arginase I in a healthy population, the contribution of hepatocyte-derived arginase I to serum arginase I may be small compared to WBC and RBC. As for uric acid, the anti-oxidative effect is well known. Uric acid is considered to be the major component in serum anti-oxidative capacity. In this study, multiple linear regression showed a significant association with arginase I only in males. Therefore, the association of arginase I with uric acid was weak.

As for the measurement of 8-isoprostane, several researchers have pointed out that mass spectrometric methods have sensitivity and specificity compared with immunoassays [45]. However, at present, many clinical researchers have employed an EIA kit for the measurement of 8-isoprostane because there were no other suitable methods for large-scale cross-sectional study besides immunoassay [46–48]. The concentrations of urinary 8-isoprostane as determined by mass spectrometric methods in human controls were reported to be in the range of 0.16–1.88 ng/mg creatinine [49–51]. Therefore, our results, in which the mean urinary 8-isoprostane was 0.69 ng/mg creatinine, did not stray from the values determined by mass spectrometric methods.

At present, there are two established methods for the measurement of urinary 8-OHdG: high-performance liquid chromatography with an electrochemical detection (HPLC-ECD) method [52] and ELISA [53]. A good correlation ($r = 0.833$; $p < 0.0001$) for urinary 8-OHdG levels was observed between these two methods [54]. Moreover, there are two commercial kits for quantifying 8-OHdG using monoclonal antibody N45.1 from the Japan Institute for the Control of Ageing (Fukuroi, Shizuoka, Japan) and another monoclonal antibody (clone 1F7) from Trevigen (Gathersburg, MD).

Although the findings are significant, several limitations should be noted in the study. First, the sample size used in this study was small. Secondly, causal relationships could not be determined because this study was a cross-sectional study. Thirdly, some reporting bias may have been introduced because the information on lifestyle factors like smoking and alcohol consumption was obtained via self-reported questionnaires. Fourthly, as there is no validated method for the measurement of urinary 8-isoprostane at the population study level, the present results should be interpreted with caution. Further studies are needed to examine the analytical method of 8-isoprostane in comparison with the 'gold standard' mass spectrometric methods and to confirm the urinary level of 8-isoprostane in healthy subjects with an increased sample size.

In conclusion, serum arginase I was associated with the urinary level of oxidative stress biomarkers such as 8-isoprostane and 8-OHdG and with serum HbA1c in addition to age, WBC and RBC in healthy Japanese people and may become a new biomarker for early prediction of diabetes mellitus and other oxidative stress-related diseases in future.

Declaration of interest

This work was supported in part by Grant-in-Aid for Science Research No. 19390163 from the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

References

[1] Jenkinson CP, Grody WW, Cederbaum SD. Comparative properties of arginase. Com Biochem Physiol B Biochem Mol Biol 1996;114:107–132.

[2] Modolell M, Corraliza IM, Link F, Solar G, Eichmann K. Reciprocal regulation of the nitric oxide synthase /arginase balance in mouse bone marrow-derived macrophages by TH1 and TH2 cytokines. Eur J Immunol 1995;25:1101–1104.

[3] Wang WW, Jenkinson CP, Griscavage JM, Kem RM, Arabolos NS, Byrns RE, Cederbaum SD, Igonarro LJ. Co-induction of arginase and nitric oxide synthase in murine macrophages activated by lipopolysaccharide. Biochem Biophys Res Commun 1995;210:1009–1016.

[4] Hey C, Boucher JL, Vadon-Le Goff S, Kettere G, Wessler I, Racke K. Inhibition of arginase in rat and rabbit alveolar macrophages by N omega-hydroxy-D, L-indospicine, effect on L-arginine utilization by nitric oxide synthase. Br J Pharmacol 1997;121:395–400.

[5] Maarsingh H, Zaagsma J, Meurs H. Arginase: a key enzyme in the pathophysiology of allergic asthma opening novel therapeutic perspectives. Br J Pharmacol 2009;158:652–664.

[6] Zimmermann N, King NE, Laprte J, Yang M, Mishra A, Pope SM, Muntel EE, Witte DP, Pegg AA, Foster PS, Hamid Q, Rotherberg ME. Dissection of experimental asthma with DNA microarray analysis identifies arginase in asthma pathogenesis. J Clin Invest 2003;111:1863–1874.

[7] Takemoto K, Ogino K, Shibamori M, Hitomi Y, Takigawa T, Wang DH, Takaki JM Ichimura H, Ishiyama H. Transiently, paralleled upregulation of arginase and nitric oxide synthase and the effect of both enzymes on pathology of asthma. Am J Physiol Lung Cell Mol Physiol 2007;293:L1419–L1426.
[8] Munder M, Mollinedo F, Calafat J, Canchado J, Gil-amaignere C, Fuentes JM, Luckner C, Doschoko G, Soler G, Eichmann K, Muller FM, Ho AD, Goerner M, Modolell M. Arginase I is constitutively expressed in human granulocytes and participates in fungicidal activity. Blood 2009;105:2549–2556.
[9] Morris CR, Poljakovic M, Lavrisha L, Mochando L, Kuypers FA, Morris SM, Jr. Decreased arginine bioavailability and increased serum arginase activity in asthma. Am J Respir Crit Care Med 2004;170:148–153.
[10] Lara A, Khatri SB, Wang Z, Comhair SA, Xu W, Dweik RA, Bodine M, Levison BS, Hammel J, Bleecker E, Busse W, Calhoun WJ, Castro M, Chung KF, Curran-Evertt D, Gaston B, Israel E, Jaejour N, Moore W, Peters SP, Teague WG, Wenzel S, Hazen SL, Erzurum SC, National Heart, Lung, and Blood Institute's Severe Asthma Research Program. Alterations of the arginine metabolome in asthma. Am J Respir Crit Care Med 2008;178:673–681.
[11] Kocyigit A, Zeyrek D, Kekes H, Koylu A. Relationship among manganese, arginase, and nitric oxide in childhood asthma. Biol Trace Elem Res 2004;102:11–18.
[12] Ceylan E, Aksoy N, Gencer M, Vyral H, Keles H, Selek S. Evaluation of oxidative-anitioxidative status and the L-arginine-nitric oxide pathway in asthmatic patients. Respir Med 2005;99:871–876.
[13] Morris CR. Asthma management: reinventing the wheel in sickle cell disease. Am J Hematol 2009;84:234–241.
[14] Morris CR, Kato GJ, Polijakovic M, Wang X, Blackweider WC, Sachdev V, Hazen SL, Vichinsky EP, Morris SM, Jr, Gladwin MT. Dysregulated arginine metabolism, hemolysis-assocated pulmonary hypertension, and mortality in sickle cell disease. JAMA 2005;294:81–90.
[15] Leu SY, Wang SR. Clinical significance of arginase in colorectal cancer. Cancer 1992;70:733–736.
[16] Rodriguez PC, Ernstoff MS, Hernndez C, Atkins M, Zabaleta J, Sieerra R, Ochoa AC. Arginase I-producing myeloid-derived suppressor cells in renal cell carcinoma are a subpopulation of activated granulocytes. Cancer Res 2009; 69:1553–1560.
[17] Wang SR, Chen ML, Huang MH, Lin HY, Tsai JJ, Kuo BIT. Plasma arginase concentration measured by an enzyme-linked immunosorbnt assay (ELISA) in normal adult population. Clin Biochem 1993;26:455–460.
[18] Maarsingh H, Zaagsma J, Meurs H. Arginine homeostasis in allergic asthma. Eur J Pahrmacol 2008;585:375–384.
[19] Munder M. Arginase: an emerging key player in the mammalian immune system. Br J Pharmacol 2009;158:638–651.
[20] Bergeron C, Boulet LP, Page N, Laviolette M, Zimmermann N, Rothernberg ME, Hamid Q. Influence of cigarret smoke on the arginase pathway in asthmatic airways: increasesd expression of arginase I. J Allergy Clin Immunol 2007;119: 391–397.
[21] Motran CC, Diaz FL, Gruppi A, Slavin D, Chatton B, Bocco JL. Human pregnancy-specific glycoprotein 1a (PSG1a) induces alternative activation in human and mouse monocytes and suppresses the accessory cell-dependent T cell proliferation. J Leukoc Biol 2002;72:512–521.
[22] Gray MJ, Poljakovi M, Kepka-Lenhart D, Morris SM, Jr. Induction of arginase I transcription by IL-4 requires a composite DNA response element for STAT6 wnd C/EBPbeta. Gene 2000;353:98–106.
[23] Halliwell B, Gutteridge JMC. Oxidative stress. In: Halliwell B, Gutteridge JMC, editors. Free radical biology and medicine. 3rd ed. New York: Oxford University Press; 1999. p 246–350.
[24] Kimura S, Yamauchi H, Hibino Y, Iwamoto M, Sera K, Ogino K. Evaluation of urinary 8-hydroxydeoxtguanosine in healthy Japanese people. Basic Clin Pharmacol Toxicol 2006; 98:496–502.
[25] Sakano N, Takahashi N, Wang DH, Sauriasari R, Takemoto K, Kannbara S, Sato Y, Takigawa T, Takaki J, Ogino K. Plasma 3-nitrotyrosine, urinary 8-isoprostane and 8-OHdG among healthy Japanese people. Free Radic Res 2009;43:183–192.
[26] Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ, 2nd. A series of prostaglangin F2-like compounds are produced *in vivo* in humans by a non-cyclooxygenase, free redical-catalyzed mechanism. Proc Natl Acad Sci USA 1990; 87:9383–9387.
[27] Helmerson J, Basu S. F2-isoprostane excretion rate and diumal variation in human urine. Prostaglandins Leukot Essent Fatty Acids 1999;61:203–205.
[28] Moller P, Loft S. Dietary antioxidants and beneficial effect on oxidatively damaged DNA. Free Radic Biol Med 2006;41: 388–415.
[29] Banerjee D, Madhusoodanan UK, Nayak S, Jacob J. Urinary hydrogen peroxide: a probable marker of oxidative stress in malignancy. Clin Chim Acta 2003;334:205–209.
[30] Yuen JW, Benzie IFF. Hydrogen peroxide in urine as a potential biomarkers of whole body oxidative stress. Free Radic Res 2003;37:1209–1213.
[31] Wang SR, Chen ML, Huang MH, Lin HY, Tsai JJ, Kuo BIT. Plasma arginase concentration measured by an enzyme-linked immunosorbant asay (ELISA) in normal adult population. Clin Biochem 1993;26:455–460.
[32] Morris CR, Suh JH, Hagar W, Laekin S, Bland DA, Steinberge MH, Vichinsky EP, Shigenaga M, Ames B, Kuypers FA, Lings ES. Erythrocyte glutamine depletion, altered redox environment, and pulmonary hypertension in sickle cell disease. Blood 2008;111:402–410.
[33] Matthiesen S, Lidemann D, Warnken M, Juergens UR, Racke K. Inhibition of NADPH oxidase by apocynin inhibits lipopolysaccharide (LPS) induced up-regulation of arginase in rat alveolar macrophages. Eur J Pharmacol 2008;579:403–410.
[34] Thengchaisri N, Hei TW, Wang W, Xu X, Li Z, Fossum TW, Kuo L. Upregulation of arginase by H2O2 impairs endothelium-dependent nitric oxide-mediated dilatation of coronary arterioles. Arterioscler Thromb Vasc Biol 2006;26:2035–2042.
[35] Muller JM, Rupec RA, Baeuerle PA. Study of gene regulation by NF-kappa B and AP-1 in response to reactive oxygen intermediates. Methods 1997;11:301–312.
[36] Gius D, Botero A, Sha S, Curry HA. Intracellular oxidation/reduction ststus in the regulation of transcription factors NF-kappaB and AP-1. Toxicol Lett 1999;106:93–106.
[37] Duffin R, Leitch AE, Fox S, Haslett C, Rossi AG. Targeting granulocyte apoptosis: mechanisms, models, and therapies. Immunol Rev 2010;236:28–40.
[38] Bayne JW, Thorpe SR. Role of oxidative stress in diabetic complications: a new perspective on an old paradigm. Diabetes 1999;48:1–9.
[39] Dandona P, Thusu S, Cook K, Synder B, Makowski J, Armstrong D, Nicotera T. Oxidative damage to DNA in diabetes mellitus. Lancet 1996;347:444–445.
[40] Brownlee M. Biochemical and molecular cell biology of diabetic complications. Nature 2001;414:813–820.
[41] Harriosn D, Griendling KK, Landmesser U, Horning B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003;91:7A–11A.
[42] Sakurai T, Tsuchiya S. Superoxide production from nonenzymatically glycated protein. FEBS Letters 1988;236: 406–410.
[43] Kaneto H, Katakami N, Atsuhisa M, Matsuoka T. Role of reactive oxygen species in the progression of type 2 diabetes and atherosclerosis. Mediat Inflamm 2010;2010:1–11.
[44] Ikemoto M, Tsunekawa S, Awane M, Fukuda Y, Murayama H, Igarashi M, Nagata A, Kasai Y, Totani M. A useful ELISA system for human liver-type arginase, and its utility in diagnosis of liver disease. Clin Biochem 2001;34:455–461.

[45] Morrow JD. Quantification of isoprostanes as induces of oxidant stress and the risk of atherosclerosis in humans. Arterioscler Thromb Vasc Biol 2005;25:279–286.
[46] Michoulas A, Tong V, Teng XW, Chang TK, Abbott FS, Farrell K. Oxidative stress in children receiving valproic acid. J Pediatr 2006; 49:692–696.
[47] Shibata H, Nabika T, Moriyama H, Masuda J, Kobayashi S. Correlation of NO metabolites and 8-iso-prostaglandin F2a with perivetricular hyperintensity severity. Arterioscler Thromb Vasc Biol 2004;24:1659–1663.
[48] Devries MC, Hamadeh MJ, Glover AW, Raha S, Samjoo IA, Tranopolsky MA. Endurance training without weight loss loweres systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women. Free Radic Biol Med 2004;45:503–511.
[49] Davi G, Guagnano MT, Ciabattoni G, Basili S, Falco A, Marinopiccoli M, Nutini M, Sensi S, Patrono C. Platelet activation in obese women: role of inflammation and oxidant stress. JAMA 2002;288:2008–2014.
[50] Barden A, Zilkens RR, Croft K, Mori T, Burke V, Beilin IJ, Puddey IB. A reduction in alcohol consumption is associated with reduced plasma F2-isoprostanes and urinary 20-HETE excretion in men. Free Radic Biol Med 2007;42: 1730–1735.
[51] Liang Y, Wei P, Duke RW, Reaven PD, Harman SM, Cutler RG, Heward CB. Quantification of 8-iso-prostaglamdin-F(2alpha) and 2,3-dinor-8-iso-prostaglndin-F(2alpha) in human urine using liquid chromatography-tandem mass spectrometry. Free Radic Biol Med 2003;34:409–418.
[52] Fenkel K, Zhoong ZJ, Wei HC, Kaekiszka J, Patel U, Rashid K, Georgescu M, Solomon JJ. Quantitative high-performance liquid chromatography analysis of DNA oxidized *in vitro* and *in vivo*. Anal Biochem 1991;196:126–136.
[53] Saito S, Yamaguchi H, Hasui Y, Kurashige J, Ochi H, Yshida K. Quantitative determination of urinary 8-hydrosydeocy-guanosine (8-OH-dg) by using ELISA. Res Commun Mol pathol Pharmacol 2000;107:1664–1670.
[54] Shimoi K, Kasai H, Yokota N, Toyokuni S, Kinae N. Comparison between high-performance liquid chromatography and enzyme-linked immunosorbant assay for the determination of 8-hydroxy-2'-deosyguanosine in human urine. Cancer Epidemiol Biomarkers Prev 2002;11:767–770.

This paper was first published online on Early Online on 28 September 2010.