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Serum Asymmetric Dimethyl-L-Arginine in Renal Failure Patients Living in Jeddah Region, Saudi Arabia

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ABSTRACT

In this study we aimed to determine the effect of gender differences on serum asymmetric dimethyl-L-arginine (ADMA) levels in renal failure patients. Serum ADMA concentrations and other factors were measured in 59 subjects, 25 control subjects (males/females 13/12) and 34 patients with renal failure (males/females 16/18). Patients attended Nephrology Department in King Abdulaziz University Hospital (Jeddah, Saudi Arabia) between December 2009 and February 2010. ADMA concentrations were measured by competitive ELISA. The serum concentrations of ADMA were significantly elevated in patients with renal failure when compared with their matched control subjects in both sexes. In males, the Mean \pm SD concentration of ADMA was 1.49 \pm 0.18 vs. 0.62 \pm 0.23 $\mu\text{mol L}^{-1}$ ($p < 0.001$). In females, ADMA concentrations were 1.31 \pm 0.27 vs. 0.59 \pm 0.16 $\mu\text{mol L}^{-1}$ ($p < 0.01$). In patients with renal failure, ADMA concentrations were significantly decreased in females as compared with males (1.31 \pm 0.27 vs. 1.49 \pm 0.18 $\mu\text{mol L}^{-1}$, $p < 0.05$). There was no significant difference in serum ADMA concentration in control male and female subjects. Thus, the present available data suggested that accumulation of ADMA showed gender differences in renal failure patients, which probably contribute to a higher risk of cardiovascular diseases and total mortality in males.

Key words: ADMA, renal failure, sex-dependent differences, cardiovascular diseases

INTRODUCTION

Cardiovascular complications are a major clinical problem in patients with renal failure; cardiac death accounts for approximately 40-50% of all deaths in these patients. Death from cardiovascular causes is up to 20 times more common in uremic patients than in the general population with the risk being even higher than in patients with diabetes mellitus (Campean *et al.*, 2005). One pathogenic mechanism that might contribute to cardiovascular risk in these patients is endothelial dysfunction. Endothelial dysfunction is characterized by reduced bioactivity of the anti-atherogenic molecule Nitric Oxide (NO) and is considered a proatherogenic condition (Cross *et al.*, 2001; Mittermayer *et al.*, 2005; Kawashima and Yokoyama, 2004; McGrowder *et al.*, 2006).

NO is the most potent endogenous vasodilator known, synthesized from L-arginine by the action of a family of NO synthase (NOS) with endothelial, neural and macrophage isoforms (Cooke, 2000; Kielstein *et al.*, 2001a; Lokhande *et al.*, 2006). Synthesis of NO can be selectively inhibited by asymmetric dimethyl-L-arginine (ADMA), a methylated L-arginine derivative, which act as a competitive inhibitor at the active site of the enzyme (Zinellu *et al.*, 2007; Brooks *et al.*, 2009).

ADMA is produced by catabolism of proteins containing methylated arginine residues via protein arginine methyl transferases and subsequently hydrolyzed (Vallance and Leiper, 2004). ADMA is eliminated in part by renal excretion. Reduced clearance of ADMA in renal failure is associated with endothelial vasodilator dysfunction, reversible by administration of L-arginine (Vallance and Leiper, 2004; Kielstein *et al.*, 2001b), or by dialysis (Boger and Zoccali, 2003). Free ADMA actively degraded by the intracellular enzyme dimethylarginine dimethylaminohydrolase (DDAH). DDAH metabolizes ADMA to L-citrulline and dimethylamine (Teerlink, 2005; Murray-Rust *et al.*, 2001).

Accumulation of ADMA has been linked to endothelial dysfunction in many diseases and repeatedly associated with kidney failure. ADMA is markedly increased in patients with renal failure, partly because of reduced renal excretion and may contribute to their excess cardiovascular event rate (Mittermayer *et al.*, 2005; Boger and Zoccali, 2003). Thus ADMA may be regarded as a uremic toxin (Kielstein *et al.*, 2001a).

High serum ADMA concentrations in renal failure patients may contribute to acceleration of cardiovascular disease and total mortality in this patient population (Boger and Zoccali, 2003; Zoungas *et al.*, 2006).

Endothelial dysfunction and reduced NO bioactivity occur in patients on haemodialysis, peritoneal dialysis and in those with chronic renal failure prior to the introduction of renal replacement therapy (Cross *et al.*, 2001; Tepel *et al.*, 2003). ADMA concentration elevated in dialyzed patients and decreased by dialysis (Boger and Zoccali, 2003). More than one-half of all dialysis-related deaths are cardiovascular in nature (Karnik *et al.*, 2001).

Since, males have a higher risk of cardiovascular diseases, the leading cause of mortality in patients with renal failure, than females; that may be due to the accumulation of ADMA in uremic males. The present study was carried out to clarify whether gender differences affect ADMA levels in renal failure patients.

MATERIALS AND METHOD

Subjects: The present study was carried out on two groups of individuals: twenty-five control subjects (13 males, 12 females); aged between 19 and 70 years and thirty-four uraemic subjects (16 males, 18 females); aged between 15 and 73 years; only 25 patients undergoing dialysis. Samples were collected from the patients during their visit to Nephrology Department in King Abdulaziz University Hospital (Jeddah, Saudi Arabia) during the period December 2009 to February 2010. A letter of consent was taken from the patients approving the collection of the samples.

Biochemical analysis: Serum ADMA levels was determined by competitive ELISA (DLD Diagnostika GmbH, Hamburg, Germany).

Sample preparation: Sample acylation was conducted in the 96-well reaction plate supplied with the kit according to the instructions of the manufacturer. Standards, kit controls and samples (20 μ L) were mixed with 25 μ L acylation buffer and 25 μ L equalizing reagent. Subsequently, 25 μ L acylation reagent was added and the reaction plate was incubated for 30 min at room temperature on an orbital shaker. Diluted equalizing reagent (100 μ L) was added and the reaction plate was again incubated for 45 min. After the incubation, the samples were ready for the ELISA analysis.

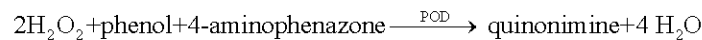
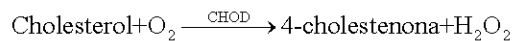
Performance of assay: The ADMA-ELISA kit consists of a split-type reaction plate (12×8) coated with ADMA, six standards (0-5 $\mu\text{mol L}^{-1}$), rabbit anti-ADMA antiserum, goat anti-rabbit-IgG-peroxidase conjugate, TMB substrate solution, stop solution and wash buffer. Aliquots (50 μL) of the acylated standards, kit controls or samples were processed according to the instructions of the kit manufacturer. Absorbance was read at 450 nm (reference wavelength 570-650 nm) using microplate photometer within 1 h. All samples, kit controls and standards were analyzed in duplicate.

Determination of clinical parameters: Serum creatinine and Blood Urea Nitrogen (BUN) were measured by using specific Flex reagent cartridges (Siemens Healthcare Diagnostics Inc., Newark, USA) on a Dimension clinical system (DADE Behring Inc., Newark, USA). Cholesterol and triacylglycerol (TAG) were determined by enzymatic analysis, according to the manufacturer's protocol (SPINREACT, Spain) using a spectrophotometer (Jenway 6305, UK).

Determination of creatinine: In the presence of a strong base such as NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 510 nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured using a bichromatic (510, 600 nm) rate technique. Bilirubin is oxidized by potassium ferricyanide to prevent interference (Knapp and Mayne, 1987).

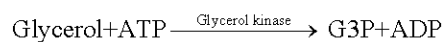
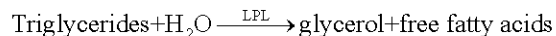
Determination of Blood Urea Nitrogen (BUN): Concentration of BUN was determined by using a bichromatic (340 nm) rate technique (Dafae *et al.*, 2008).

Determination of cholesterol: The cholesterol present in the sample originates a colored complex, according to the following reaction:



The intensity of the color formed is proportional to the cholesterol concentration in the sample (Natio and Kaplan, 1984; Meattini *et al.*, 1978).

Determination of TAG: TAG is measured according to the following reaction:



The intensity of the color formed is proportional to the triglycerides concentration in the sample (Bucolo and David, 1973; Fossati and Prencipe, 1982).

Statistical analysis: The data are expressed as Means±SD comparison between mean of two groups was performed using independent-sample t-test. Statistically significant results were considered at $p < 0.05$ or less. Pearson correlation coefficients (r) were used to assess the significance of the association of ADMA with serum creatinine, BUN, Cholesterol and TAG. The statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL), version 12.0 for windows.

RESULTS AND DISCUSSION

Table 1 shows clinical data of control and renal failure subjects. Control and uremic subjects were well matched for age. Mean serum ADMA concentration was significantly higher in patients than in control subjects in both sexes. In uremic males, ADMA concentrations was 1.49 ± 0.18 vs. $0.62 \pm 0.23 \mu\text{mol L}^{-1}$ in the control ($p < 0.001$) and 1.31 ± 0.27 vs. $0.59 \pm 0.16 \mu\text{mol L}^{-1}$ ($p < 0.01$) in females subjects. Significant increases in serum cholesterol, creatinine and urea were observed in the males with renal failure when compared with control (cholesterol: 3.26 ± 1.82 vs. $2.12 \pm 0.76 \text{ mmol L}^{-1}$ ($p < 0.05$); creatinine, 500.88 ± 280.18 vs. $79.69 \pm 20.11 \mu\text{mol L}^{-1}$ ($p < 0.001$); urea, 18.84 ± 11.32 vs. $4.46 \pm 1.39 \text{ mmol L}^{-1}$ ($p < 0.001$)). In females, significant increases were found in the concentrations creatinine and urea (creatinine: 455.50 ± 165.47 vs. $55.83 \pm 14.18 \mu\text{mol L}^{-1}$ ($p < 0.001$); urea, 11.93 ± 7.87 vs. $3.64 \pm 2.25 \text{ mmol L}^{-1}$ ($p < 0.001$)). There were no significant associations among age and TAG in both sexes.

Male subjects show higher serum ADMA levels than female subjects. In patients with renal failure, ADMA concentration was significantly decreased in females as compared with males ($1.31 \pm 0.27 \mu\text{mol L}^{-1}$ vs. $1.49 \pm 0.18 \mu\text{mol L}^{-1}$, $p < 0.05$). There was slightly but not significantly increases in ADMA level in control males than females.

Correlations of factor potentially associated with ADMA in renal failure patients are showed in Table 2. In males with renal failure, a positive correlation were found between serum ADMA and cholesterol ($r = 0.524$, $p < 0.05$), creatinine ($r = 0.541$, $p < 0.05$, Fig. 1) and urea ($r = 0.508$, $p < 0.05$, Fig. 2a). No correlations were found with age or TAG. In uremic females, ADMA was inversely correlated with cholesterol ($r = 0.498$, $p < 0.05$) and urea ($r = -0.591$, $p < 0.01$, Fig. 2b). No correlations were found between ADMA and age, TAG or creatinine.

Table 1: Clinical characteristics of subject studies

Variable	Males		Females	
	Control (13)	Renal failure patients (16)	Control (12)	Renal failure patients (18)
Age (years)	53.69±18.38	54.75±22.60 ^{ns}	43.50±26.81	40.67±20.44 ^{ns}
Cholesterol (mmol L ⁻¹)	2.12±0.76	3.26±1.82*	3.45±1.52	2.45±0.94 ^{ns}
TAG (mmol L ⁻¹)	1.24±0.99	1.65±1.69 ^{ns}	1.05±0.58	0.75±0.38 ^{ns}
Creatinine (μmol L ⁻¹)	79.69±20.11	500.88±280.18***	55.83±14.18	455.50±165.47***
Urea (mmol L ⁻¹)	4.46±1.39	18.84±11.32***	3.64±2.25	11.93±7.87***
ADMA (μmol L ⁻¹)	0.62±0.23	1.49±0.18***	0.59±0.16	1.31±0.27***

Results are expressed as Mean±SD, with (n) is the number of subjects. Significant differences between control and other groups are shown as: * = $p < 0.05$, *** = $p < 0.001$, ns: non-significant

Table 2: Bivariate analysis of factor potentially associated with ADMA in renal failure patients

	Males		Females	
	r	p	r	p
Age	0.011	0.967 ^{ns}	-0.301	0.225 ^{ns}
Cholesterol	0.524	0.037*	-0.498	0.036*
TAG	0.168	0.534 ^{ns}	-0.154	0.542 ^{ns}
Serum Creatinine	0.541	0.031*	-0.334	0.175 ^{ns}
Urea	0.508	0.045*	-0.591	0.010**

*Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed), ns: non significant

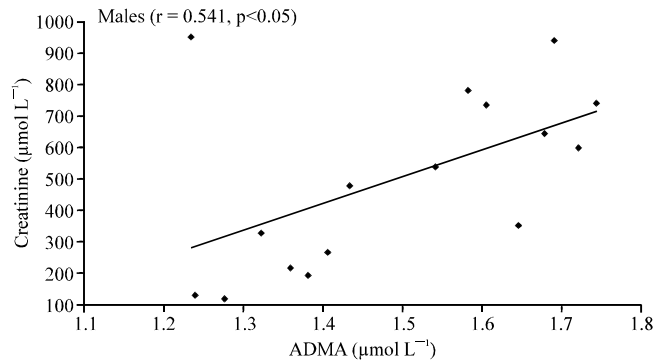


Fig. 1: Correlation analysis between ADMA levels and creatinine in male patients with renal failure

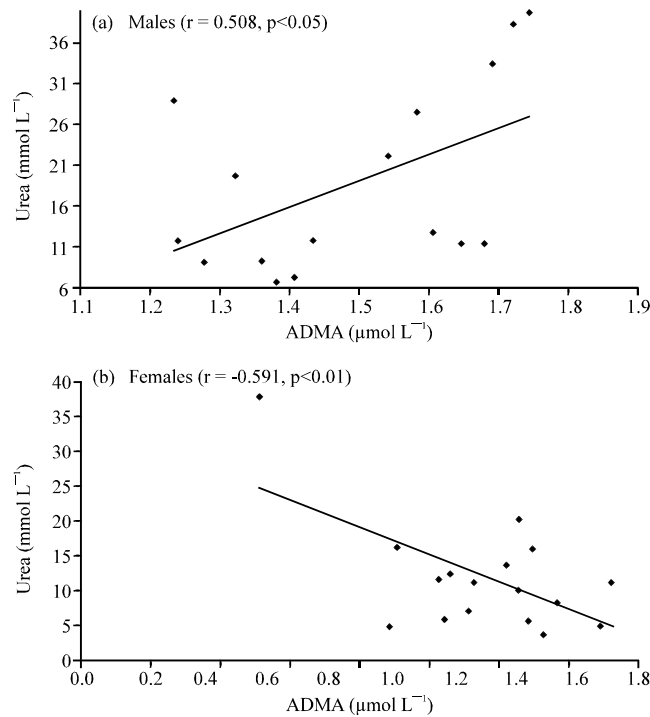


Fig. 2: Correlation analysis between ADMA levels and urea in (a) male and (b) female patients with renal failure

The results of this study demonstrate that sex-dependent differences were found in serum ADMA concentration in controls and uremic patients, ADMA concentrations were significantly higher in patients with renal disease compared with matched controls, increased serum creatinine levels associated with increased levels of ADMA in uremic males and elevated levels of ADMA might contribute to greater risk for cardiovascular diseases in uremic males than females.

ADMA correlated with several risk factors in the absence of clinical disease (Miyazaki *et al.*, 1999). It is predictive for the all over mortality and cardiovascular outcome in renal failure patients. The mortality rate in these patients is 10%/yr (Zoccali *et al.*, 2001). ADMA are markedly increased at a very early stage of renal failure, even when Glomerular Filtration Rate (GFR) is still within the normal range (Kielstein *et al.*, 2002).

There is substantial agreement that the plasma concentration of ADMA is higher in patients with uremia than in subjects with normal renal function (Vallance *et al.*, 1992; MacAllister *et al.*, 1996; Kielstein *et al.*, 1999; Zoccali *et al.*, 2001, 2002). In this study, control males had non significant increase in serum ADMA levels when compared to females. In patients with renal failure, males showed significantly higher ADMA levels than females. This may explain why men are at great risk for coronary heart disease (Fodor and Tzerovska, 2004; Rgitz-Zagrosek *et al.*, 2006).

The accumulation of endothelial toxins in uremia could contribute to the impaired NO-mediated dilatation reported in renal failure patients (Vallance *et al.*, 1992). NO deficiency due to inhibited production by ADMA, impairs cardiac function and increases peripheral vascular resistance (Lin *et al.*, 2002; Kielstein *et al.*, 2004; Achan *et al.*, 2003). Thus, ADMA is a biologically active NOS inhibitor with a long duration of action (Fliser *et al.*, 2005). Chronically elevated ADMA blood levels may promote progression of renal (vascular) disease via endothelial damage as a consequence of reduced NO availability (Fliser *et al.*, 2005; Erdely *et al.*, 2003; Kang *et al.*, 2002). The high incidence of hypertension and atherosclerosis encountered in patients with terminal renal failure might be caused by accumulation of ADMA as a result of reduced renal excretion which is sufficiently high to reduce significantly NO production (Vallance *et al.*, 1992; Xiao *et al.*, 2001).

In this study the mean age for females (control: 43.50±26.81, uremic: 40.67±20.44 years) was younger than males (control: 53.69±18.38, uremic: 54.75±22.60 years) and females showed lower serum ADMA levels than males. This result was in agreement with other published data (Dammati and Khoja, 2009; Schulze *et al.*, 2005) which found sex-dependent differences between ADMA and age. Females younger than 50 years had lower serum ADMA levels than males but higher levels with the onset of menopause and this may be explained by differences in hormonal status.

In humans, approximately 300 µmol of ADMA is generated per day, approximately 250 µmol of which is metabolized by the enzyme DDAH, whereas only a minor amount is excreted unchanged by the kidneys (Achan *et al.*, 2003). Metabolism of ADMA by DDAH may also be impaired in uremia (Kielstein *et al.*, 1999; Kielstein *et al.*, 2001b). DDAH is found in a variety of human tissues, including kidney, pancreas and human blood vessels (Najbauer *et al.*, 1993; MacAllister *et al.*, 1996). A high DDAH activity has been observed in the kidney (Leiper *et al.*, 1999; Tran *et al.*, 2000) which have revealed that the kidney is a major extraction site for ADMA from the circulation (Nijveldt *et al.*, 2002). These data may explain why even minor renal dysfunction leads to accumulation of ADMA (Fliser *et al.*, 2005).

In the presence of an increased serum creatinine and urea or a decreased GFR, morbidity and mortality because of CVD are markedly increased and vascular degradation is accelerated (Aronson *et al.*, 2008; Vanholder *et al.*, 2005). In this study, males had a higher serum creatinine

and urea than females in control and uremic subjects. Males with renal failure showed a positive correlation between serum ADMA and creatinine ($r = 0.541$, $p < 0.05$), urea ($r = 0.508$, $p < 0.05$). Thus, increased levels of ADMA in males may relate to increased levels of serum creatinine and urea. In uremic females, unexpected result was obtained. There was no correlation between ADMA and creatinine and ADMA was inversely correlated with urea ($r = -0.591$, $p < 0.01$).

In conclusion, accumulations of ADMA are correlated to gender differences in patients with renal failure. Thus, ADMA may be considered as an independent predictor of future cardiovascular events and total mortality in these patients.

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