Plasma asymmetric dimethylarginine and its association with some of cardiovascular disease risk factors in chronic kidney disease

Tam Vo, MBBS[1], Thang Hoang Viet, MBBS[1], Quoc Hoang Trong Ai, MBBS[1,2]

[1]Department of Nephrology, Hue University of Medicine and Pharmacy, 06 Ngo Quyen Street, Hue city, Vietnam, [2]Department of Emergency Medicine, Hue Central Hospital, Hue City, Vietnam

ABSTRACT

Introduction: Chronic kidney disease (CKD) usually has increase of asymmetric dimethylarginine (ADMA) levels. ADMA is a cardiovascular disease (CVD) risk factor and its elevation associated with other CVD risk factors at CKD leads to increasing risk of death. In this article, we aimed to identify levels and elevation proportion of plasma ADMA in CKD as well as association between ADMA with CVD risk factors.

Methods: This cross-sectional study was performed at Hue Central Hospital from 2012-2016 on 176 CKD and 64 control subjects. ADMA levels were measured by using the enzyme linked immunosorbent assay (ELISA) method.

Results: Mean ADMA level was markedly higher (p<0.001) in all patients combined (0.73±0.24μmol/L) than in control subjects (0.47±0.13μmol/L). Mean ADMA levels in advanced kidney disease were higher than control subjects. ADMA levels correlated inversely and relatively strictly to estimated glomerular filtration rate (eGFR) (r = -0.689; p<0.001), haemoglobin (r = -0.525; p<0.001) and haematocrit (r = -0.491; p<0.001); correlated favourably and relatively strictly to serum creatinine (r = 0.569; p<0.001) and serum urea (r = 0.642; p<0.001). ADMA elevation was predicted simultaneously by eGFR<60 mL/min/1.73m^2 (p<0.001), anaemia (p=0.002), body mass index (BMI) (p=0.011) and high sensitivity C-reactive protein (hs-CRP) (p=0.041). Cut-off of ≥0.68μmol/L, ADMA levels predict reduction of eGFR<60 mL/min/1.73m^2, sensitivity of 86.9 %, specificity of 82.6%, area under ROC 92.4% (95%CI: 88.6-96.1%).

KEY WORDS:
CKD, ADMA, CVD risk factor, stage, correlation

INTRODUCTION

Prevalence and incidence of chronic kidney disease (CKD) is increasing worldwide.[1,2] It often has an association between CKD and cardiovascular disease (CVD). Most of CKD patients died from CVD, and CKD patents usually have elevated asymmetric dimethylarginine (ADMA) levels. This substance has biological activities by inhibition and regulation of nitric oxide activity. Nitric oxide plays an important role in vascular endothelial cells. Reduction of nitric oxide activity may cause endothelial damage and other consequences.[3] There were many researches that found association between ADMA levels and CVD. Elevated ADMA levels lead to increasing risk of CVD as well as death in general population and CKD patients.[4-6] Therefore, elevated ADMA level is an important non-traditional CVD risk factor of CKD.

There were studies of ADMA levels on CKD, however, few of them evaluated ADMA levels at all stages of CKD. Evaluation of association between ADMA levels and CVD risk factors had different results.[4,7-10] So, we performed this study at 176 non-diabetic CKD patients in different stages in order to find levels and proportion of elevation of plasma ADMA in CKD as well as associations between ADMA levels with some of CVD risk factors.

MATERIALS AND METHODS

Patients and control subjects

The protocol was approved by the ethics committee of Hue University of Medicine and Pharmacy and Hue Central Hospital. All participants gave informed consent.

One hundred seventy-six adults with chronic glomerulonephritis and chronic stone interstitial nephritis were confirmed by history, ultrasound, x-ray and biological investigations. Chronic glomerulonephritis was diagnosed by renal biopsy. Criteria of CKD was based on 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.[11] All of the participants were conservatively treated at the outpatient and inter-hospital departments.

In all patients, a detailed history and a thorough physical examination, including electrocardiogram, were obtained to exclude relevant disease. Patients with known diabetes mellitus of any type, liver disease, malignancy, thyropathy, infectious disease, immunosuppressive therapy and a history of alcohol abuse were excluded from the study. Hypertension was defined as blood pressure (BP) >140/90mmHg and/or on anti-hypertensive medication.[12] Anaemia were according to World Health Organization criteria.[13] Cardiovascular risk factors were based on the Statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and

This article was accepted: 19 March 2019
Corresponding Author: Dr. Quoc Hoang Trong Ai
Email: aiquochue@yahoo.com

Epidemiology and Prevention.[14] This statement indicated kidney disease as a risk factor for development of cardiovascular disease. Chronic kidney disease is also associated with increased prevalence of traditional and non-traditional cardiovascular disease risk factors.

All patients examined were assigned to one of five stages with respect to their eGFR based serum creatinine estimated by EPI-CKD 2009 count (Chronic Kidney Disease Epidemiology Collaboration – 2009), staged by the National Kidney Foundation criteria 2012,[11] as following: stage 1, above 90mL/min/1.73 m^2 (normal or high); stage 2, between 60.0-89.9 mL/min/1.73m^2 (slight reduction); stage 3, between 30.0-59.9mL/min/1.73m^2; stage 4, between 15.0-29.9 mL/min/1.73m^2 and stage 5, under 15.0mL/min/1.73m^2.

Sixty-four healthy adults were included as controls, consisted of age-, sex-, and BMI-matched volunteers without a history of kidney disease, CVD or diabetes, and with normal urinalysis and urine sediment. All participants were in stable condition for at least three months before the initiation of the study, and their comorbidities related to endothelial dysfunction (hypertension, ischemic heart disease, peripheral arterial disease) were effectively treated.

Blood samples for measurement of routine chemistry, haemoglobin (Hb), serum creatinine, hs-CRP, serum lipoprotein cholesterol and ADMA levels were taken after an overnight fast of at least 12h. The samples were centrifuged immediately at 1500 x g and 4°C for 10 min. The sample of ADMA was stored in at -20°C until further use.

Measurements and Calculations

Plasma concentrations of ADMA were measured by the method of competitive enzyme linked immunoassays (ELISA) with kits of Immundiagnostik AG (ADMA ELISA Kit), Germany. After taking 2mL venous fasting blood, the sample was centrifuged, and the plasma was storage at -20°C until quantification. The treated samples and the polyclonal ADMA-antiserum are incubated in wells of a microtiter plate coated with ADMA-derivative (tracer). During the incubation period, the target ADMA in the sample competes with the tracer immobilised on the wall of the microtiter wells for the binding of the polyclonal antibodies. The ADMA in the sample displaces the antibodies out of the binding to the tracer. Therefore, the concentration of the tracer-bound antibody was inverse proportional to the ADMA concentration in the sample. Determination of absorption was done immediately with a photometer reader at 450nm. A dose response curve of absorbance unit vs. concentration was generated using the values obtained from the standard. Cut-off of elevated plasma ADMA level was considered as plasma mean value + 2 x standard deviation (X + 2SD).

All other measurements, including serum creatinine, hs-CRP, lipoprotein cholesterol and Hb were performed using routine laboratory tests and certified methods. eGFR using EPI-CKD 2009 count was assessed in all patients.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows 18.0. Continuous variables were compared between groups with unpaired t-test or the nonparametric Wilcoxon rank sum test as appropriate. Dichotomized variables were compared using Pearson chi-square test. The null hypothesis was rejected at $p<0.05$. Data are presented as mean ± SD if variables were standard distribution. In case of distribution was not standard, a presentation of median (25%; 75% interval) was used. Univariate correlation was performed by Spearman correlation analysis. Regression correlation model was organised and a ROC curve was draw to predict a phenomenon.

RESULTS

Table I shows clinical data of patients with CKD and control subjects. Mean plasma ADMA concentration was markedly higher in patients than in the control subjects. There was almost no overlap of ADMA values between both groups, so that the difference between the combined results of all patients (0.73±0.24µmol/L) and control subjects (0.47±0.13µmol/L) was highly significant ($p<0.001$). Proportion of plasma ADMA level elevation of CKD group was significantly higher than of control group (OR = 52.2, 95%CI: 7.12-387.02, $p<0.001$).

Plasma ADMA levels were similar in control group and stage 1 CKD patients. However, these levels in other stages were higher significantly in comparison to the control subjects. ADMA concentration was also significantly higher in the group with advanced kidney failure in comparison to former group (Figure 1).

Our study found no association between ADMA level and gender.

With cut-off ≥0.73µmol/L as elevation of ADMA level, our study showed that there was a high ADMA elevation rate in CKD group vs. control group (OR: 52.5, 95%CI: 7.12-387.02, $p<0.001$) and so in anaemia CKD vs. non-anaemia CKD (OR: 11.16, 95%CI: 5.12- 24.34, $p<0.001$) (Table II).

Consequently, the correlation between plasma ADMA concentrations and eGFR in CKD group was high ($r = -0.689$, $p<0.001$) (Figure 1); the correlation between plasma ADMA concentrations and Hb in CKD group was high too ($r = -0.525$, $p<0.001$), and so were the correlations between ADMA levels and age ($r = 0.225$, $p<0,01$), mean arterial BP ($r = 0.16$, $p<0.05$), BMI ($r = -0.35$, $p<0.001$), serum TG ($r = 0.149$, $p<0.05$). There were not the correlations between ADMA levels and serum hs-CRP, and serum cholesterol, and serum HDLC, and serum LDLC ($p>0.05$) but serum TG ($p<0.05$) (Table III). With cut-off of plasma ADMA levels ≥0.7µmol/L, it existed anaemia with sensitivity of 72.0%, specificity of 81.2%, area under ROC curve of 82.2% (95%CI: 76.1%-88.3%). With cut-off of ≥0.68µmol/L, ADMA levels predict reduction of eGFR<60 mL/min/1.73m2, sensitivity of 86.9 %, specificity of 82.6%, area under ROC curve 92.4% (95%CI: 88.6%-96.1%). There was multivariable regression model between ADMA with BMI ($p<0.05$), creatinine ($p<0.01$) and eGFR ($p<0.001$) and an inverse regression correlation between eGFR and ADMA ($t = -57.278$; $p<0.001$), age ($t = -627$; $p<0.001$), mean BP ($t = -0.320$; $p<0.05$), serum TG ($t = -2.482$; $p<0.05$) and serum creatinine ($t = -0.032$; $p<0.001$);

Table I: Clinical data of control subjects and patients with CKD

Parameter	Control subjects	Patients with CKD				
		Stages of CKD				
No. patients	64	32	37	30	33	44
Gender (M/F)	30/34	18/14	18/19	20/10	19/14	18/26
Age (yr)	51±19	40±16	54±14	60±18	64±19	55±17
S-Creat. (μmol/L)	73.66±12.05	72.94±11.52	86.15±17.20	135.5±34.21	232.9±51.78	886.29±551.60
eGFR (mL/min/1.73 m^2)	96.05±18.45	105.5±14.12	74.76±8.93	46.04±10.23	22.53±4.23	6.11±3.25
BMI (kg/m2)	21.02±2.90	21.20±2.75	20.73±2.25	21.22±3.00	19.53±2.98	18.74±2.20
Systolic BP (mmHg)	118.52±11.40	132.19±23.10	134.46±25.49	141.00±28.57	141.21±21.14	151.45±32.42
Diastolic BP (mmHg)	73.91±6.58	81.72 ±11.54	81.08 ±13.08	82.67±13.63	81.03 ±10.43	85.77±13.68
Mean BP (mmHg)	88.78±7.58	98.54±14.39	98.87±16.49	102.11±17.39	101.09±12.54	107.83±19.05
ADMA (μmol/L)	0.47± 0.13	0.52± 0.13	0.59± 0.10	0.68± 0.11	0.83±0.13	0.97±0.26
hs-CRP (mg/l)	1.00					
(0.44 - 3.28)	2.14					
(0.53 - 22.69)	8.86					
(1.6 - 28.65)	5.22					
(1.61 - 76.48)	8.20					
(1.49 - 26.80)	6.35					
(1.21 - 28.03)						
Hb (g/l)	127.89±19.06	133.78±15.41	124.89±16.38	125.60±21.57	105.24±21.23	83.70±21.49
Hct (%)	38.88±5.27	39.26±8.39	37.46±5.19	37.26±3.96	31.69±6.18	25.34±6.64
Chol. (mmol/l)	4.91±1.26	5.77±2.33	5.30±1.45	5.45±2.86	5.88±2.97	5.05±1.29
TG (mmol/d)	1.67±1.07	1.97±1.35	1.97±2.03	2.63±2.74	2.40±1.77	2.08±0.97
HDLC (mmol/l)	1.44±0.63	1.41±0.38	1.42±0.44	1.21±0.39	1.35±0.48	1.29±0.92
LDLC (mmol/l)	2.77±1.11	3.54±2.08	3.13±0.87	3.30±2.57	3.65±2.57	3.04±1.03

S-creat., serum creatinine concentration; eGFR, estimated glomerular filtration rate; BMI, body mass index; BP, blood pressure; ADMA, plasma asymmetric dimethylarginine concentration; hs-CRP, high-sensitive C reactive protein; Hb, hemoglobin; Hct, hematocrit; Chol., total serum cholesterol concentration; TG, serum triglyceride concentration; HDLC, high-density lipoprotein cholesterol concentration; LDLC, low-density lipoprotein cholesterol concentration

Table II: ADMA level elevation in CKD and control, anaemia CKD and non-anaemia CKD

ADMA elevation*		No	Yes	OR (95%CI)	p
CKD group	n	80	96	52.5 (7.12-387.02)	<0.001
	%	45.5	54.5		
Control group	n	63	1		
	%	98.4	1.6		
Anaemia CKD	n	37	70	11.16 (5.12-24.34)	<0.001
	%	43.6	65.4		
Non-anaemia CKD	n	59	10		
	%	85.5	14.5		

* Cut-off value of ADMA level elevation: 0.73μmol/L

Table III: Correlation between ADMA levels and CVD risk factors

	Correlation	r	p
ADMA (μmol/L)	Age (y)	0.225	<0.01
	Mean BP (mmHg)	0.160	<0.05
	BMI (kg/cm^2)	- 0.350	<0.001
	Hb (g/L)	- 0.525	<0.001
	Chol (mmol/L)	- 0.052	>0.05
	HDLC (mmol/L)	-0.143	>0.05
	TG (mmol/L)	0.149	<0.05
	LDLC (mmol/L)	-0.065	>0.05
	hs-CRP (mg/L)	-0.015	>0.05

Table IV: Logistic regression between ADMA elevation and BMI, hypertension, eGFR reduction, hs-CRP, anaemia

Index	Constant	OR	95% CI	p
BMI (kg/m^2)	-0.212	0.809	0.686-0.953	<0.05
Hypertension	-0.913	0.401	0.13 -1.198	>0.05
eGFR<60 mL/min/1.73m^2	3.709	40.811	11.401-146.091	<0.001
hs-CRP (mg/L)	-0.009	0.991	0.982-1.000	<0.05
Anaemia	1.575	4.829	1.753-13.301	<0.01

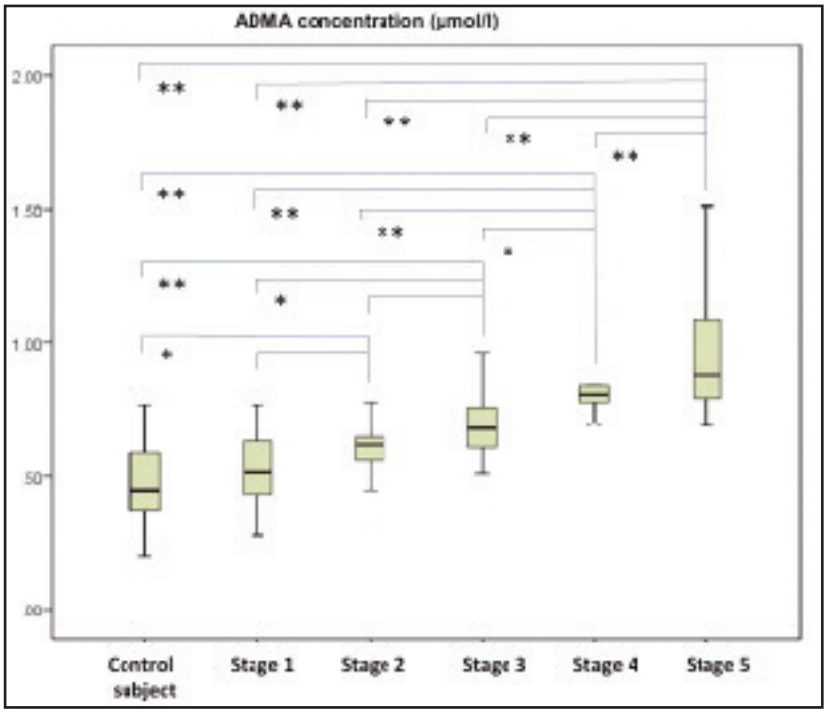

Fig. 1: Box plots of plasma ADMA concentrations in CKD patients with stage 1 (n = 32; GFR 105.5±14.12 mL/min/1.73 m^2; ADMA 0.52±0.13 µmol/L), stage 2 (n = 37; GFR 74.76±8.93 mL/min/1.73 m^2; ADMA 0.59±0.10 µmol/L), stage 3 (n = 30; GFR 46.04±10.23 mL/min/1.73 m^2; ADMA 0.68±0.11 µmol/L), stage 4 (n = 33; GFR 22.53±4.23 mL/min/1.73 m^2; ADMA 0.83±0.13 µmol/L), stage 5 (n = 44; GFR 6.11±3.25 mL/min/1.73 m^2; ADMA 0.97±0.26 µmol/L). Mean ADMA concentration were higher, particularly in patients with advanced kidney dysfuncrion. *p<0.01; **p<0.001.

favourable regression correlation between eGFR and Hb (t = 0.220; p<0.05). There was a logistic regression between ADMA elevation and BMI, eGFR reduction, hs-CRP, anaemia (Table IV).

DISCUSSION

Plasma ADMA levels of control subjects and CKD group

Many researches of ADMA in CKD had a high ADMA levels as well and our results concur with other existing studies.

Mean of CKD ADMA levels with eGFR<90 mL/min/1.73m^2 in our research was similar to ones in many researches,15 Tetty Hendrawati's research[18] (18) and Paola Pecchini's research[19] (19). All utilised ELISA method to quantify ADMA levels.

Danilo Fliser's research presented lower ADMA levels than our results and above authors. This research utilised HPLC-MS.[7] Jan T. Kielstein reported a mean of plasma ADMA levels higher than our research's and other researches.[9] Lise Tarnow with research in diabetic CKD presented mean of ADMA levels lower than above researches.[20] Both utilised HPLC method.

With eGFR<60 mL/min/1.73m^2, Jaromír Eiselt showed the same of ADMA levels to our results (p>0.05).[10] With eGFR ≥30µmol/L, our ADMA levels was higher than ADMA levels in Prabath W.B. Nanayakkara's research (p<0.01).[21]

Generally, our mean of ADMA levels concurred to levels from researches that utilized ELISA. This method used monoclonal antibody and it was simpler than HPLC in getting reproducible results. Tetty Hendrawati's research and ours had same proportion of ADMA elevation (p = 0.362).

The most striking finding of this study is the demonstration that, in CKD patients without diabetes, plasma concentrations of the endogenous NO synthase inhibitor ADMA, a putative biochemical marker of endothelial dysfunction, atherosclerosis and kidney disease[3], are markedly increased at an early stage when there is a mild reduction of eGFR. Simultaneously, there was an increase of ADMA elevation proportion associated with eGFR reduction. Many researches also presented plasma ADMA levels in different stages. In which, our ADMA levels with CKD stage 3 were similar to levels in Tri P. Asmarawati's research. Our ADMA levels were also similar to levels in Tetty Hendrawati's research at stage 3-4 and in Mac Allister's research at stage 5. Our results were significantly higher than Danilo Fliser's results at all stages, Tri P. Asmarawati's results at stage 5 and significantly lower than Tetty Hendrawati's ones at stage 2.[7,18,22,23]

In Tetty Hendrawati's study, ADMA levels of CKD stage 5 were lower than ADMA levels of CKD stage 4. This was explained by the author that there was an increase of homocysteine and S-adenosylhomocysteine (SAH) intracellular level. Since SAH is a potent inhibitor for

transmethylation reactions, its accumulation can result in macromolecules hypomethylation. This leaded to decrease free ADMA levels.[18]

Our study showed an increase of plasma ADMA level in CKD and the results mainly contributed by the advanced CKD patients. With levels of ADMA ≥0.73µmol/L, there were 45.5% of CKD had elevation of ADMA in comparison to 1.6% of control subjects (p<0.001). Generally, proportion of ADMA elevation in CKD was higher than 50 folds to control subjects (OR = 52.5; p<0.001) (Table II). This showed that there was a big difference of ADMA levels in CKD patients and healthy persons.

A meta-analysis based on participants with pre-existing renal diseases found that circulating ADMA showed a predictive value for mortality.[6] Previous studies presented that a high circulating ADMA concentration was inversely related to glomerular filtration rate and positively correlated with progression to dialysis.[15,16] Increased ADMA levels predicted higher risk for cardiovascular occurrence (20% per 0.1µmol/L increase in plasma level).[15] Although there was recent study on its structural isomer symmetric dimethylarginine (SDMA) presenting that SDMA predicts CKD progression and future atherosclerotic cardiovascular events more consistently than other methylarginines, it needs more studies to evaluate role of this isomer.[17]

Relationship between plasma ADMA levels and some of CVD risk factors

Correlation between ADMA and age found our research was reported by Danilo Fliser (r = 0.596; p<0.01)7 and so by Lise Tarnow.[20] However, this correlation was not found by Jan T. Kielstein (2002) in CKD stage 1-49 by Prabath W.B. Nanayakkara21 and by other researchers.[24-26]

The correlation between ADMA and age was different in each research. This was probably that ADMA concentrations were affected by many factors in which change of GFR was a reason. In study of Jan T. Kielstein, there were only 44 CKD for stages. So, it was difficult to evaluate the correlation.

Obesity caused risk of GFR reduction. Many researches presented a higher BMI of CKD to control subjects.[9,10,27] However, our research showed an inverse result. It was explained by malnutrition in ESKD. It also had an increase of ADMA associated with GFR reduction. These factors leaded to negative correlation in this research.

Hypertension is a major risk factor for cardiovascular and kidney disease. Conversely, CKD with ADMA increase, reduced generation of vasodilators such as nitric oxide is the most common form of secondary hypertension and mounting evidence suggests it is an independent risk factor for cardiovascular morbidity and mortality.[28] In our study, there was positive correlation between ADMA levels and mean arterial pressure. However, this correlation was not strong (Table III). This could be explained by that there are many factors affecting on hypertensive mechanism in CKD. So, in some cases, role of ADMA on hypertension is not strong enough and it can lead to a mild or no correlation between ADMA and BP such as in this study.

Anaemia is common in CKD. It is also a non-traditional CVD risk factor. Our research presented a significantly higher ADMA of anaemia CKD to non-anaemia CKD (0.82±0.24µmol/L; 0.59±0.13µmol/L, respectively, p<0.001). Proportion of elevated ADMA in anaemia CKD was higher than 11 folds to non-anaemia CKD (OR = 11.16; 95%CI: 5.12-24.34; p<0.001) (Table II). Bing Chang Vincent Lau reported that OR of anaemia (Hb<10g/L) was similar in stage 1 and 2. However, OR increased to 16,76 folds higher in stage 5.[29] Correlation between ADMA and Hb concentrations in our research was negatively strong (r = -0,525, p<0,001). This association reported by Danilo Fliser (2005)[7] was moderate level (r = -0.336; p<0.001). So, report of Kaan Gökçen was similar (r = -0.292; p=0.018)(24). Anaemia and CKD were independently associated with a 100% increase of death.[30] So, this correlation could cause a higher CVD risk to our CKD patients. However, study of Miyuki Yokoro[31] presented that it was erythrocyte ADMA levels significantly correlated with haemoglobin levels (r = -0.41, p=0.002), whereas plasma ADMA levels showed no association with haemoglobin levels. It also showed that plasma and erythrocyte ADMA levels were not correlated with each other in patients with CKD. Erythrocyte ADMA accumulation played a role in impaired response to Epo in a mouse model of advanced CKD. However, in this study, 50.0% of patients were diabetes mellitus comparing to our study of 100% non-diabetic CKD.

According to Shankar Anoop (2011), elevation of CRP levels may be a marker/intermediary effect of antecedent inflammatory processes (e.g., TNF, a mediated inflammation). As a result, CRP elevations may be evident only in relatively later stages of kidney disease development, and therefore not useful for early prediction of CKD. Alternatively, it is also possible that any CRP–CKD association may entirely be explained by confounders that were adjusted in our multivariable model, such as age, gender, BMI, diabetes, and hypertension.[32] So, it was not surprise that there was not association between ADMA and hs-CRP concentrations in our research.

CKD results in dysregulation of lipid metabolism, especially HDLC and TG, which contributes to arteriosclerotic CVD and possibly accelerated progression of kidney disease. However, this association is not always strictly. Hence, our research found no correlation between ADMA and total cholesterol, HDLC and LDLC, exception of TG.

Multivariable regression model between ADMA with GFR and BMI was explained as a result from mechanism of ADMA elevation and malnutrition in advanced CKD.

In relationship between ADMA level elevation and anthropometry, clinic and paraclinic factors in CKD, the result showed that eGFR<60 mL/min/1.73 m^2 (p<0.001) and anaemia (p<0.01) predict better than BMI and hs-CRP (p<0.05).

In relationship between eGFR and ADMA, age, mean BP, BMI, creatinine, Hb, TG in CKD, factors of ADMA, age and serum creatinine predicted better than mean BP, Hb, and TG. In evaluation of ADMA elevation, eGFR<60 mL/min/1.73 m^2 (p<0.001) and anaemia (p<0.01) predicted this status better than BMI and hs-CRP (p<0.05).

CONCLUSION

Plasma ADMA levels in CKD were increasing with deterioration of kidney function. Proportion of plasma ADMA level elevation was highest at stage 5. Its levels related to some of traditional and non-traditional CVD risk factors. It is necessary to have more researches to find underlying possible reason factors in relationship between ADMA levels and CVD risk factors in CKD.

ACKNOWLEDGEMENTS

We would like to thank the following persons for their contribution in design and data collection: Dr. Phuong Le Thi Dieu (Biochemistry Department, Hue Central Hospital); Dr. Anh Lê Thị Phương (Biochemistry Department, Hue Central Hospital). We would also like to thank to colleagues at many departments of Hue Central Hospital for administration and contribution in the analysis and interpretation of the data.

REFERENCES

1. "USRDS annual data report: Epidemiology of kidney disease in the United States" [Internet]. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD. 2014 [cited 31/12/2015]. Available from: http://www.usrds.org.
2. Zhang QL, Rothenbacher D. Prevalence of chronic kidney disease in population-based studies: systematic review. BMC Public Health 2008; 8: 117.
3. Aldámiz-Echevarría L, Andrade F. Asymmetric dimethylarginine, endothelial dysfunction and renal disease. Int J Mol Sci 2012; 13(9): 11288-311.
4. Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M et al. Asymmetric dimethylarginine and cardiovascular risk: systematic review and meta-analysis of 22 prospective studies. J Am Heart Assoc 2015; 4(6): e001833.
5. Tripepi G, Mattace Raso F, Sijbrands E, Seck MS, Maas R, Boger R et al. Inflammation and asymmetric dimethylarginine for predicting death and cardiovascular events in ESRD patients. Clin J Am Soc Nephrol 2011 Jul; 6(7): 1714-21.
6. Zhou S, Zhu Q, Li X, Chen C, Liu J, Ye Y et al. Asymmetric dimethylarginine and all-cause mortality: a systematic review and meta-analysis. Sci Rep 2017; 7: 44692.
7. Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Böger SM, Haller H, et al. Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. J Am Soc Nephrol 2005; 16(8): 2456-61.
8. Christian F, Schweitzer F, Karge E, Busch M, Stein G. Serum concentrations of asymmetric (ADMA) and symmetric (SDMA) dimethylarginine in patients with chronic kidney diseases. Clin Chim Acta 2003; 336(1-2): 1-12.
9. Kielstein JT, Boger RH, Bode-Boger SM, Frölich JC, Haller H, Ritz E et al. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol 2002; 13(1): 170-6.
10. Eiselt J, Rajdl D, Racek J, Vostrý M, Rulcová K, Wirth J. Asymmetric dimethylarginine and progression of chronic kidney disease: a one-year follow-up study. Kidney Blood Press Res 2014; 39(1): 50-7.
11. National Kidney Foundation. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International 2013; 3(1): 1-150.
12. Mancia G, Fagard R, Narkiewicz K, Redón J, Zanchetti A, Böhm M et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013; 31(7): 1281-357.
13. World Health Organization. Haemoglobin concentration for the diagnosis of anaemia and assessment of severity. WHO/NMH/NHD/MNM/11.1 2011.
14. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, Hamm LL et al. Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 2003; 108(17): 2154-69.
15. Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetrical dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: a competing risks modeling approach. J Am Soc Nephrol 2005; 16(8): 2449-55.
16. Lu TM, Chung MY, Lin CC, Hsu CP, Lin SJ. Asymmetric dimethylarginine and clinical outcomes in chronic kidney disease. Clin J Am Soc Nephrol 2011; 6(7): 1566-72.
17. Emrich IE, Zawada AM, Martens-Lobenhoffer J, Fliser D, Wagenpfeil S, Heine GH et al. Symmetric dimethylarginine (SDMA) outperforms asymmetric dimethylarginine (ADMA) and other methylarginines as predictor of renal and cardiovascular outcome in non-dialysis chronic kidney disease. Clin Res Cardiol 2018; 107(3): 201-13.
18. Hendrawati T, Bakri S, Arif M. The analysis of asymetric dimethylarginine and homocysteine in patients with chronic kidney disease. The Indonesian Biomedical Journal 2009; 1(2): 64-73.
19. Pecchini P, Malberti F, Mieth M, Quinn R, Tripepi G, Mallamaci F et al. Measuring asymmetric dimethylarginine (ADMA) in CKD: a comparison between enzyme-linked immunosorbent assay and liquid chromatography-electrospray tandem mass spectrometry. J Nephrol 2012; 25(6): 1016-22.
20. Tarnow L, Hovind P, Teerlink T, Stehouwer CD, Parving HH. Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in type 1 diabetes. Diabetes Care 2004; 27(3): 765-9.
21. Nanayakkara PW, Teerlink T, Stehouwer CD, Allajar D, Spijkerman A, Schalkwijk C et al. Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure. Kidney Int 2005; 68(5): 2230-6.
22. Asmarawati TP, Widodo, Thaha M, Aditiawardana, Mardiana N, Ardhany AR et al. Comparison of asymmetric dimethylarginine levels between stages three, four, and five non-dialysis of chronic kidney disease. Acta Med Indones 2016; 48(1): 28-34.
23. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E. Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure. Nephrol Dial Transplant 1996; 11(12): 2449-52.
24. Gökçen K, Kılıçarslan H, Coşkun B, Ersoy A, Kaygısız O, Kordan Y. Effect of ADMA levels on severity of erectile dysfunction in chronic kidney disease and other risk factors. Can Urol Assoc J 2016; 10(1-2): E41-5.
25. Madenci ÖÇ, Yucel N, Arikan Z, Sargin M, Akbaba D, Kaya O et al. The relationship between glycemic control and asymmetrical dimethylarginine levels. Turkish Journal of Biochemistry 2013; 38(3): 286-90.
26. Young JM, Terrin N, Wang X, Greene T, Beck GJ, Kusek JW et al. Asymmetric dimethylarginine and mortality in stages 3 to 4 chronic kidney disease. Clin J Am Soc Nephrol 2009; 4(6): 1115-20.
27. Chen J, Hamm LL, Mohler ER, Hudaihed A, Arora R, Chen CS et al. Interrelationship of multiple endothelial dysfunction biomarkers with chronic kidney disease. PLoS One 2015; 10(7): e0132047.
28. Tedla FM, Brar A, Browne R, Brown C. Hypertension in chronic kidney disease: navigating the evidence. Int J Hypertens 2011; 2011: 132405.
29. Lau BC, Ong KY, Yap CW, Vathsala A, How P. Predictors of anemia in a multi-ethnic chronic kidney disease population: a case–control study. Springerplus 2015; 4: 233.
30. Fishbane S. Anemia, chronic kidney disease and cardiovascular disease: the clinical trials. Advanced Studies in Medicine 2005; 5(7A): S715-20.
31. Yokoro M, Nakayama Y, Yamagishi SI, Ando R, Sugiyama M, Ito S et al. Asymmetric dimethylarginine contributes to the impaired response to erythropoietin in CKD-Anemia. J Am Soc Nephrol 2017; 28(9): 2670-80.
32. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. Kidney Int 2011; 80(8): 806-21.