Comparison of Asymmetric Dimethylarginine Levels Between Stages Three, Four, and Five Non-dialysis of Chronic Kidney Disease

Tri P. Asmarawati, Widodo, M. Thaha, Aditiawardana, Nunuk Mardiana, Ardityo R. Ardhany, Artaria Tjempakasari, Djoko Santoso, Pranawa, Chandra Irwanadi

Department of Internal Medicine, Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

Corresponding Author:
M. Thaha, Prof, MD, PhD. Department of Internal Medicine, Faculty of Medicine, Airlangga University. Jl. Mayjen. Prof. dr. Moestopo 6-8, Surabaya 60131, Indonesia. email: tripudyasmarawati@gmail.com, mochthaha@yahoo.com.

ABSTRAK

Tujuan: *mengetahui perbandingan kadar asymmetric dimethylarginine (ADMA) antar-stadium 3, 4, dan 5 pasien penyakit ginjal kronik (PGK) non-dialisis di RSUD Dr. Soetomo Surabaya.* ***Metode:*** *penelitian potong lintang dilakukan di Instalasi Rawat Jalan Ginjal dan Hipertensi RSUD Dr. Soetomo mulai bulan Januari-Februari 2015. Stadium PGK ditentukan berdasarkan rumus perkiraan LFG menurut rumus MDRD 4 variabel. Analisis statistik perbedaan kadar ADMA ketiga kelompok subyek menggunakan uji Anova satu arah.* ***Hasil:*** *sebanyak 75 subyek, terdiri atas masing-masing 25 pasien PGK Stadium 3, 4, dan 5 non-dialisis. Umur rerata pasien PGK stadium 3, stadium 4, dan stadium 5 non-dialisis masing-masing 57,12 tahun, 54,80 tahun, dan 53,68 tahun. Rerata kadar ADMA pada kelompok stadium 3 adalah 0,62 (0,11) IU/mL, kelompok stadium 4 adalah 0,72 (0,16) IU/mL, dan kelompok stadium 5 adalah 0,73 (0,18) IU/mL. Terdapat perbedaan kadar ADMA secara bermakna (p=0,04) antar-stadium PGK, dengan perbedaan terbesar pada perbandingan kelompok stadium 3 dan 5.* ***Kesimpulan:*** *terdapat perbedaan kadar ADMA yang bermakna antar-stadium PGK dan semakin tinggi pada stadium PGK yang lebih tinggi.*

Kata kunci: *penyakit ginjal kronis, asymmetric dimethylarginine (ADMA), penyakit kardiovaskular.*

ABSTRACT

Aim: *to determine the differences of ADMA level between stages 3, 4, and 5 non-dialysis of chronic kidney disease (CKD) patients at Outpatient Nephrology Clinic, Dr. Soetomo Hospital.* ***Methods:*** *a cross-sectional study was conducted on stage 3, 4, and 5 non-dialysis CKD patients at Outpatient Nephrology Clinic, Dr. Soetomo Hospital, Surabaya from January to February 2015. Stages of CKD were determined based on GFR estimation according to 4-variable MDRD formula. Statistical analysis of differences in the levels of ADMA in three subject groups use one-way ANOVA test.* ***Results:*** *seventy-five patients were included in the study. Each group consisted of 25 patients stage 3, 4, and, 5 non-dialysis patients. Mean age of stage 3, stage 4, and stage 5 non-dialysis CKD patients were respectively 57.12 years, 54.80 years and 53.68 years. The mean levels of ADMA in stage 3, stage 4, and 5 were 0.62 (0.11) IU/mL, 0.72 (0.16) IU/mL, and 0.73 (0.18) IU/mL respectively. Analysis of the differences between the groups showed significant differences in ADMA levels (p=0.04), with the highest difference between stage 3 and stage 5.* ***Conclusion:*** *comparison of ADMA levels showed significant differences between CKD stages and the level tends to be higher along with increase severity of CKD stages.*

Keywords: *chronic kidney disease, asymmetric dimethylarginine (ADMA), cardiovascular disease.*

INTRODUCTION

Cardiovascular disease (CVD) and atherosclerotic complications are the most important causes of morbidity and mortality in patients with chronic kidney disease (CKD).[1] Some evidences of association between renal dysfunction and cardiovascular complications was first discovered in dialysis population, where the incidence of cardiovascular mortality was very high. It was also found in subjects with less severe impaired renal function.[2] Traditional risk factors and non-traditional risk factors such as inflammation, oxidative stress, endothelial dysfunction, coronary artery calcification, hyperhomocysteinemia, and immunosuppressants have been associated with accelerated atherosclerosis. Coronary artery disease (CAD) is one of the primary types of CVD in patients with CKD. Atypical presentation of CAD in patients with CKD often leads to delay in diagnosis and treatment. The diagnostic value of baseline ECG is often limited by non-specific changes due to left ventricular hypertrophy and electrolyte disturbances. Furthermore, the outcomes of CAD are poorer in patients with CKD than non-CKD counterparts.[3]

Endothelial dysfunction is common in CKD patients. It is due to the reduced bioavailability of nitric oxide (NO). Nitric oxide has a protective role for the cardiovascular system because it inhibits vascular muscle cell proliferation, platelet aggregability, and the adhesion of monocytes to the endothelium. Asymmetrical dimethylarginine (ADMA) is an endogenous inhibitor of endothelial nitric oxide synthase (eNOS) which may contribute to endothelial dysfunction and may participate actively in development of atherogenesis in patients with end-stage renal disease. ADMA is in part excreted by the kidney, but this compound is mainly disposed by transformation into citrulline, a reaction driven by the enzyme diethyl-diamino-hydrolase (DDAH). Diethyldiaminohydrolase is present within the endothelial cells and is very sensitive to oxidative stress. Oxidative stress is pervasive in ESRD, therefore, high ADMA in this condition may be the expression of the high rate of generation of oxidants.[4]

Studies have demonstrated that high ADMA is strongly associated with well established risk markers such as increased intima-media thickness in the carotid arteries and concentric left ventricular hypertrophy.[5-8] ADMA predicts death and cardiovascular complications independently of other risk factors in end-stage renal disease (ESRD) patients.[9] Therefore, measuring the plasma ADMA level may be important to determined the cardiovascular risk in patient with CKD and manage the atherosclerotic complication earlier.

Previous researches showed that in patients with mild to moderate CKD, ADMA level is inversely correlated with glomerular filtration rate (GFR) and is a strong predictor for the progression towards ESRD and death in CKD patients underwent coronary angiography.[9,10] Study performed by Kielstein et al.[11] meanwhile showed that plasma level of ADMA are not correlated with GFR. Another study showed that mean plasma ADMA levels were not significantly different in renal patients with normal GFR and mild reduction of GFR but were significantly higher with more advanced stages of renal failure.[12] However, most of the previous studies performed also in CKD patient with CVD comorbidity and traditional risk factors whereas the elevation of ADMA level might be related to the CVD rather than the severity of CKD. The previous studies also did not analyze the differences of the ADMA level according to the CKD stages. An estimated GFR below 60 ml/min/1.73 m^2 is a risk factor for both new and recurrent cardiovascular disease in the general population and in people at increased risk for cardiovascular disease. In these patients, cardiovascular morbidity is more common than progression to kidney failure.[13] We conducted this study to determine and analyze the differences of the ADMA level in CKD patient with GFR less than 60 ml/min/1.73 m^2. Comparing the differences of ADMA level between stages of CKD especially in moderate to advanced stage could provide us information about which stadium should undergo early intensive evaluation and treatment of risk factors for cardiovascular disease.

METHODS

Cross-sectional study was conducted to examine ADMA levels at various CKD stages

at Outpatient Nephrology Clinic, Dr. Soetomo Hospital, Surabaya. Data collection was done from January to March 2015. The inclusion criteria were patients with CKD stage 3, 4, and 5 non-dialysis aged 21-65 years, and agreed to participate in the study. The exclusion criterias were subjects with cigarette smoking, poorly controlled diabetes mellitus, uncontrolled hypertension, history of cardiovascular disease, multiple organ failure, presence of hepatic dysfunction, pregnant, and on antioxidant use since these condition influenced the ADMA level. We determined the sampel size by equation for unpaired numerical analytical study and the result was 25 in each group, so that the total sample in three groups comprised of 75 patients.[14] Sampling was done using retrieval technique with consecutive sampling system.

CKD stage classification was based on NFK-KDOQI Guidelines in 2002 and the determination of the estimated glomerular filtration rate (eGFR) use the 4-variables modification of diet in renal disease (MDRD) formula.[15] Patients with CKD stages 1 and 2 were not included because these groups were difficult to find in nephrology outpatient clinic. Non-dialysis stage 5 CKD patients in this study were patients with eGFR<15 ml/min/m^2 but not yet experiencing symptoms of uremia. Determination of ADMA use ADMA ELISA kit made by standardized laboratory. Concentration in the plasma was determined in units of µmol/L.[16] Based on internal studies in healthy subjects, the average levels of ADMA was 0.45 µmol/l. Normal ADMA level ranges was average serum/plasma ± 2 SD = 0.45 ± 0.19 µmol/l.[17]

All of the subjects who met the inclusion and exclusion criteria were recorded for demography and clinical characteristics which include age, gender, body mass index, blood pressure, history of antihypertensives (ACE-inhibitor, ARB) medications, and antidiabetic drugs (metformin, thiazolidinediones, insulin). Venous blood sampling was performed on each subject for examination of ADMA levels, BUN, and serum creatinine. All data collected were then analyzed with a statistical test to determine differences in ADMA levels between the three groups of samples.

Data processing was done by the program R version 3.2.[18,19] The data were analyzed with descriptive statistics and presented in the form of a frequency distribution table. Statistical analysis of ADMA levels differences among the three groups of subjects used one-way ANOVA test when data distribution was normal and data variance was the same as well. Post hoc analysis is conducted when the result of ANOVA showed significant differences between at least two groups to determine the magnitude of the differences between each group.[14]

RESULTS

The total number of samples in this study were 75 patients with CKD who were divided into 3 groups of stages, each consist of 25 patients. Demographic and clinical characteristics of the subjects are shown in **Table 1**. The mean age of the study subjects was 55.20 (8.16) years. Parameters of hemoglobin and albumin showed that the higher the CKD stage of the patients, the lower the average value obtained.

Table 2 shows the average levels of ADMA between stage groups. Post Hoc analysis are shown in **Table 3**, in which there is the highest difference in stages 3 and 5, while the lowest difference was in stage 4 and 5.

DISCUSSION

CKD has become recognised as a key independent risk factor for CVD. It is now increasingly apparent that individuals are more likely to die from cardiovascular disease than to develop ESRD. Initial evidence indicating a relationship between renal dysfunction and adverse cardiovascular events become apparent in those on dialysis, where the number of CVD deaths was found to be raised.[20] Furthermore, CVD in CKD patients, as the major cause of death, cannot be entirely explained by the traditional cardiovascular risk factors. It has been hypothesized that this excessive risk can be attributed, at least in part, to endothelial dysfunction and reduced bioavailability of nitric oxide (NO), which might play a pivotal role in the initiation and progression of atherosclerosis and might be a potential link between cardiovascular disease and CKD.[9]

Table 1. Demography and clinical characteristics of the subjects

Characteristics	Stage 3 (n=25)	Stage 4 (n=25)	Stage 5 non-dialysis (n=25)
Age (years), mean (SD)	57.12 (5.40)	54.80 (7.14)	53.68 (10.89)
Sex (male), n (%)	16 (64.0)	8 (32.0)	11 (44.0)
CKD Causes, n (%)			
- DM	6 (24.0)	10 (40.0)	10 (40.0)
- Non-DM	19 (76.0)	15 (60.0)	15 (60.0)
Proteinuria degree, n (%)			
- +1	16 (64.0)	13 (52.0)	3 (12.0)
- +2	7 (28.0)	8 (32.0)	11 (44.0)
- +3	1 (4.0)	2 (8.0)	6 (24.0)
- +4	1 (4.0)	2 (8.0)	5 (20.0)
Estimated GFR (mL/min/1.73m^2), mean (SD)	40.34 (7.45)	22.22 (3.84)	10.55 (3.21)
Serum Creatinine (mg/dl), median (range)	1.73 (1.20-2.40)	2.68 (1.90-3.70)	5.79 (3.30-15.50)
Albumin (g/L), mean (SD)	4.54 (0.35)	4.23 (0.45)	4.09 (0.49)
Haemoglobin (g/dL), mean (SD)	12.92 (1.96)	11.30 (2.02)	10.13 (1.50)
DM, n (%)			
- Insulin use	0 (0.0)	3 (33.3)	5 (50.0)
- Tiazolidindion use	0 (0.0)	2 (20.0)	1 (10.0)
- Metformin use	0 (0.0)	0 (0.0)	0 (0.0)
Random Blood Glucose (mg/dl), mean (SD)	109.88 (34.45)	107.92 (29.44)	116.36 (32.37)
Hypertension, n (%)			
- ACEI/ARB use	11 (44.0)	11 (44.0)	12 (48.0)
- Non HT/ non ACEI/ARB	14 (56.0)	14 (56.0)	13 (52.0)
Systolic BP (mmHg), mean (SD)	133.16 (14.52)	131.44 (16.32)	139.72 (13.12)
Diastolic BP (mmHg), mean (SD)	80.04 (9.45)	75.68 (10.36)	74.48 (11.21)
Dyslipidemia, n (%)			
- Statin use	8 (32.0)	10 (40.0)	4 (16.0)
- Non-dyslipidemia/ non-statin	17 (68.0)	15 (60.0)	21 (84.0)
LDL (mg/dl), mean (SD)	102.28 (28.71)	109.08 (29.48)	93.32 (28.23)

Table 2. ADMA level between CKD stages

CKD groups	Total	Mean (SD)	p value
Stage 3	25	0.63 (0.11)	0.04
Stage 4	25	0.72 (0.16)	
Stage 5	25	0.73 (0.18)	

Table 3. Results of post-hoc analysis

	Mean Difference	Min	Max	p value
Stage 4 vs 3	0.09	-0.01	0.19	0.10
Stage 5 vs 3	0.10	-0.00	0.20	0.06
Stage 4 vs 5	0.01	-0.09	0.11	0.97

Asymmetric dimethylarginine is inhibitor of endothelial NOS. In those with ESRD, ADMA has been independently associated with the intima-media thickness of the carotid artery and may predict its progression during a one-year follow-up period.[4,6] In a cohort of hemodialysis patients, scientists found that plasma ADMA may be a strong and independent risk factor of total mortality and cardiovascular outcome.[20] Asymmetric dimethylarginine is thought to be significantly associated with the oxidative stress process through its inhibition of NO, and thus leading to endothelial dysfunction and vascular damageand plays a major role in potentiating atherosclerosis.[4]

Asymmetric dimethylarginine levels are markedly elevated in renal impairment. Several prospective studies showed association between ADMA and CKD progression. Each 0.1 μmol/l increase of ADMA may increase 47% progression

of CKD.[12] A study in patients with type 1 diabetes and overt diabetic nephropathy showed that an ADMA level higher than the median was associated with a faster yearly decline in GFF and a greater risk of developing ESRD over a median follow-up of 11.3 years.[21] A study in patients with type 2 diabetes (with normoalbuminuria or microalbuminuria) followed up over 5.2 years reported progression of diabetic nephropathy to a more advanced stage.[22] Patients with ADMA levels above the median had a 2.7-fold higher risk of disease progression. Together, these studies provide strong evidence that increased ADMA levels are associated with progression of CKD.[23]

NO is synthesized by stereospecic oxidation of the terminal guanidino nitrogen of the amino acid L-arginine by the action of NOS. NO synthesis can be selectively inhibited by competitive blockade of the NOS active site with guanidino-substituted analogues of L-arginine such as ADMA. ADMA is released from proteins that have been post-translationally methylated, and subsequently hydrolysed. These proteins are largely found in the nucleolus and appear to be involved in RNA processing and transcriptional control. There are two types of enzymes that methylate arginine residues: protein arginine methyl-transferase type I (PRMT I) forms ADMA and LNMA, whereas PRMT II forms symmetric dimethylarginine (SDMA), that is a stereoisomer of ADMA, which has no direct inhibitory effect on NOS. ADMA is renally excreted to some extent, but the major metabolic pathway is degradation by the enzyme DDAH, which hydrolyses ADMA to dimethylamine and L-citrulline. There are two isoforms of DDAH: DDAH I is predominately found intissues that express neuronal NOS, whereas DDAH II is predominately found in tissues expressing endothelial NOS. The increased ADMA levels that inhibit NOS resulting in decreased NO levels, which has major adverse consequences for the kidney and might lead to progression of CKD.[24-26]

Genetic predisposition factors are associated with increased ADMA in CKD. Dimethylarginine dimethylaminohydrolase-2 gene variations and PMRT-1 gene polymorphism are associated with ADMA levels in hemodialysis patients.[27] The decline in DDAH-2 enzyme activity may inhibit ADMA metabolism and PMRT-1 increased activity will lead to increased production of ADMA, so both have a role in the increase of ADMA in CKD.[28]

Altough ADMA level was found to be elevated in CKD, the correlation between ADMA level and GFR showed conflicting results. In this study it was found that there was a significant difference in the levels of ADMA between CKD stages, with the lowest level in CKD stage 3 and the highest level was in CKD stage 5 (p=0.04). Post hoc analysis showed that the highest difference between two groups was found in the comparison of CKD stages 3 and 5, while the least difference was in the comparison between CKD stage 4 and 5. A study conducted by Ronden RA et al[29] examined the decline in renal clearance and increased plasma ADMA levels in hypertensive subjects with mild to moderate renal insufficiency. The results of this study showed similar trends with our study, but the ADMA level for eGFR group 30-59 was lower than ADMA level in group stages 3 and 4 in our study. The difference is probably because the subjects included in Ronden's study were hypertensive patients, instead of CKD patients as in our study. Another explanation is that there is difference in ADMA measurement methods. Another study conducted by Eloot, et al which aims to determine whether the value of eGFR describes uremic toxins, including ADMA concentrations, at various stages of CKD, also showed similar results with our research.[30]

Previous studies showed that in patients with mild to advanced CKD, ADMA level is inversely correlated with GFR and it is a strong predictor for the progression towards ESRD and death.[9,10] A study conducted by Kielstein et al.[11] showed that levels of ADMA increased independently of renal function in patients with CKD and did not correlate with GFR. Other studies in patients with mild to moderate kidney disease (GFR 30-90) indicates that the decline in GFR is associated with increased ADMA levels, but decrease in clearance of plasma ADMA is not associated with increased ADMA levels.[29] Our study results showed that there was narrow differences of ADMA level in CKD stage 4 and 5 while in CKD

stage 3 and 4 had wider differences. This suggest that ADMA level started to increased in patient with mild to moderate CKD and reach the highest level in ESRD. However, in advanced CKD the elevation are not always correlate with GFR. This also suggest that there may be other mechanisms besides the influence of GFR that plays a role in ADMA accumulation in CKD.

However, our study has several limitations. The staging of CKD was based on estimated GFR instead of measured GFR and conducted only in one time measurement. We did not involve all CKD stages in our study, so that the pattern of ADMA accumulation in all stages could not be analyzed. The causes of CKD was only distinguished by DM and non-DM, whereas other causes of CKD, such as stones, infections, and autoimmune diseases, were not detailed. Some confounding variables were obtained only from very subjective history taking.

CONCLUSION

The mean level of ADMA in group stages 3, 4 and 5 were respectively 0,63 μmol/L, 0.72 μmol/L and 0.73 μmol/L. There were differences in ADMA level between CKD stages and ADMA levels increase along with the higher stage of CKD. The greatest increase in ADMA levels was between CKD stage 3 and stage 4, so that any attempt to reduce cardiovascular complications should be done at the time or before it reaches stage 3.

REFERENCES

1. Amaresan MS. Cardiovascular disease in chronic kidney disease. Indian J Nephrol. 2005;15:1-7.
2. Schiffrin EL, Lipman ML, Mann JFE. Chronic kidney disease: Effects on the cardiovascular system. Circulation. 2007;116:85-97.
3. Cai Q, Mukku VK, Ahmad M. Coronary artery disease in patients with chronic kidney disease: A clinical update. CurrCardiol Rev. 2013;9:331-9.
4. Landim MBP, Casella Filho A, Chagas ACP. Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis. Clinics. 2009;64(5):471-8.
5. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, Imaizumi T. Endogenous nitric oxide synthase inhibitor: A novel marker of atherosclerosis. Circulation. 1999;99:1141-6
6. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Böger R. CREED investigators: asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol. 2002;13:490-6.
7. Alsagaff MY, Thaha M, Aminuddin M, Yogiarto RM, Yogiantoro M, Tomino Y. Asymmetric dimethylarginine: a novel cardiovascular risk factor in end-stage renal disease. J Intern Med Res. 2012; 40:340-9.
8. Selcoki Y, Aydin M, Ikizek M, Armutcu F, Eryonucu B, Kanbay M. Association between asymmetric dimethylarginine and the severity of coronary artery disease in patients with chronic kidney disease. Turk Neph Dial Transpl. 2011;20(1):58-64.
9. Lu TM, Chung MY, Lin CC, Hsu CP, Lin SJ. Asymmetric dimethylarginine and clinical outcome in chronic kidney disease. Clin J Am SocNephrol. 2011; 6:1566-72.
10. Ravani P, Tripepi G, Malberti F, Testa S, Mallamaci F, Zoccali C. Asymmetric dimethylarginine predicts progression to dialysis and death in patients with chronic kidney disease: A competing risks modeling approach. Am J Soc Nephrol. 2005; 24:2449-55.
11. Kielstein JT, Boger RH, Bode-Boger SM, Frolich JC, Haller H, Ritz E, Fliser. Marked increase of asymmetric dimethylarginine in patients with incipient primary chronic renal disease. J Am Soc Nephrol. 2002;13: 170–6.
12. Fliser D, Kronenberg FT, Morath KJ, Bode-Boger C, Haller H. Asymmetric dimethylarginine and progression of chronic kidney disease: The mild to moderate kidney disease study. J Am Soc Nephrol. 2005;16:2456-61.
13. Sarnak MJ, Levey AS, Schoolwerth AC, 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 andPrevention. Hypertension 2003;42:1050-65.
14. Rosner B. Fundamentals of biostatistics. 7th ed. Boston: Brooks/Cole, Cengage Learning; 2010. p. 222.
15. Kidney Disease Improving Global Outcome. KDIGO clinical practice guideline for evaluation and management of CKD. Kidney Int. 2012;3(1).
16. Blackwell S. The biochemistry, measurement and current clinical significance of asymmetric dimethylarginine. Ann Clin Biochem. 2010;47:17-28.
17. Schulze F, Wesemann R, Schwedhelm E, et al. Determination of asymmetric dimethylarginine (ADMA) using a novel ELISA assay. Clin Chem Lab Med. 2004;42(12):1377–83.
18. Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant. 2013;48:452–8.
19. Fox J, Valat MB. Getting started with the R commander. 2014.

20. Zoccali C, Bode-Böger S, Mallamaci F, et al. Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. Lancet. 2001;358:2113-7.
21. Lajer M. Plasma concentration of asymmetric dimethylarginine (ADMA) predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. Diabetes Care. 2007;31:747–52.
22. Hanai K. Asymmetric dimethylarginine is closely associated with the development and progression of nephropathy in patients with type 2 diabetes. Nephrol Dial Transpl. 2009;24:1884–8.
23. Kronenberg F. Emerging risk factors and markers of chronic kidney disease progression. Nat Rev Nephrol. 2009;5:677-89.
24. Kielstein JT, Frolich JC, Haller H, Fliser D. ADMA (Asymmetric Dimethylarginine): an atherosclerotic disease mediating agent in patients with renal disease? Nephrol Dial Transplant. 2001;16:1742-5.
25. Schwedhelm E, Böger RH. The role of symmetric and asymmetric dimethylarginine in renal disease. Nat Rev Nephrol. 2011;7:275–85.
26. Ueda S, Yamagishi SI, Okuda S. New pathways to renal damage: role of ADMA in retarding renal disease proression. J Nephrol. 2010;23(4):377-86.
27. Thaha M, Yusuf M, Yogiarto RM, Yogiantoro M, Handayani R. Polymorphism of DDAH2 gene and its association with ADMA level in CKD-5D patients. J Nephrol. 2013;3:75-81.
28. Thaha M, Nilamsari WP, Yusuf M, Amin M. Profile of PRMT-1 gene polymorphism in hemodialysis patients with increased ADMA level. Acta Med Indones. 2014;46(2):97-103.
29. Ronden RA, Houben AJHM, Teerlink T, et al. Reduced renal plasma clearance does not explain increased plasma asymmetric dimethylarginine in hypertensive subjects with mild to moderate renal insufficiency. Am J Physiol Renal Physiol. 2012;303:149-56.
30. Eloot S, Schepers E, Barreto DV, et al. Estimated glomerular filtration rate is a poor predictor of concentration for a broad rangge of uremic toxins. Clin J Am Soc Nephrol. 2011;6:1266-73.