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DIFFERENCES OF ASYMMETRIC DIMETHYL ARGININE LEVEL IN PATIENTS WITH DIABETIC NEPHROPATHY AND NON-DIABETIC NEPHROPATHY

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ABSTRACT

Endothelial dysfunction occurs early in Diabetic Nephropathy (DN), characterized by elevated Asymmetric Dimethylarginine (ADMA) levels. Increased ADMA levels may inhibit endothelial Nitric Oxide Synthase (eNOS) production which are required for Nitric Oxide (NO) formation. Decreased NO levels can increase peripheral resistance and exacerbate the endothelial dysfunction. By knowing the difference of ADMA levels in DN and non-DN patients can help the follow-up and management for the progression of endothelial dysfunction. The purpose of this research was to know the difference of ADMA levels in DN and non-DN by a cross-sectional observational analytical method in 53 diabetes mellitus patients at the Dr. Hasan Sadikin Hospital Bandung (December 2016-July 2017). Urine samples were examined to calculate urinary creatinine albumin ratio (uACR) and serum for ADMA levels. Asymetric dimethylarginin was examined by micro ELISA. Most of the subjects were males (60.38%) with the highest age in the range of 55-64 years (45.28%). Increased ADMA levels were found in 100% of DN and 18.5% of non-DN. Median ADMA levels were found in DN 1.01(0.73-2.25) µmol/L and non-DN 0.57(0.27-1.17) µmol/L, showing a significant difference of ADMA levels ($p<0.001$). High ADMA levels showed endothelial dysfunction in DN. Serum ADMA levels in DN patients were higher than in non-DN.

Key words: Asymmetric dimethylarginine, diabetic nephropathy, non-diabetic nephropathy, urinary albumin creatinine ratio

INTRODUCTION

Diabetic Nephropathy (DN) is one of the most common microvascular complications in patients with Diabetes Mellitus (DM) and ranks second after hypertension as a cause of End-Stage Renal Disease (ESRD). Endothelial dysfunction of the glomerulus occurs through several cellular mechanisms that progress on the breakdown of the glomerular basement membrane and podocytes.[1] Clinically, DN is characterized by persistent albuminuria (>30 mg/24 h), decreased Glomerular Filtration Rate (GFR) and increased blood pressure. The urinary albumin-creatinine ratio (uACR) is a gold standard of DN diagnosis according to PERKENI 2015.[2] Diabetics have a tendency for DN reinforced by unmodified (as genetic, racial, age) and modified risk factors (as hypertension, dyslipidemia, obesity, and smoking).[3] Some journals include risk factors such as dyslipidemia and obesity (asessed with Body Mass Index (BMI) as having an effect on increased ADMA' slevels.

Dyslipidemia may cause an increased risk of cardiovascular disease. A study showed that the administration of statin drugs (HMG CoA reductase inhibitor) in patients with DM can reduce the incidence of vascular disease and reduce the rate of GFR decline.4

Diabetic mellitus increased in proportion to IMT increase, by 2-8 times of BMI 25 kg/m2 and increased 1-40 times at a BMI of >30, and more, but this depends on age, sex ethnicity, and distribution of adiposity. Obesity is a factor that plays a role in the increased incidence of DM, especially DMT2. Weight gain and lack of physical activity cause insulin resistance. Increased IMT signifies the presence of fatty tissue especially in visceral organs. Fat metabolism will produce free fatty acids, leptin hormones, and various pro-inflammatory cytokines (adipokines) especially TNF-a, IL-6 which can trigger the occurrence of insulin resistance as the basic pathogenesis of DMT2.5

Endothelial dysfunction occurs early in the course of renal complications due to chronic hyperglycemia characterized by elevated levels of Asymmetric dimethylarginine (ADMA). Increased ADMA levels may inhibit the production of endothelial Nitric Oxide Synthase (eNOS) needed to help the formation of NO as an endothelial vasodilator. Decreased NO production can lead to vasoconstriction, increased peripheral resistance, glomerular hypoperfusion that will exacerbate the state of endothelial dysfunction.[6,7]

Asymmetric dimethylarginine is a major inhibitor of endogenous eNOS with 202 Da molecular weight that is produced continuously produced protein metabolism of the cell nucleus during the protein turnover process. After the process of proteolysis, ADMA compounds will be found in the cytoplasm and can be detected in the blood circulation. In healthy individuals, the average levels of ADMA synthesized within 24 hours reach 300 µmol and 250 µmol is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) and only 50 µmol is excreted via the urine. DDAH enzymes largely mediates ADMA degradation, so when there is an inhibition of the enzyme, it causes an increase

The objective of this study was to analyze the differences in ADMA levels in association with obesity (BMI) and dyslipidemia as risk factors in diabetic nephropathy and non-diabetic nephropathy.

METHODS

This study was an observational analytical study with a cross-sectional study design in diabetic nephropathy, and non -diabetic nephropathy patients. The subjects of the study were patients who had been diagnosed clinically and laboratory as DM in the Endocrine clinic of Hasan Sadikin Hospital, and clinical Pathology Laboratory in June-July 2017. Patients willing to participate in this research received explanation, and signed an informed consent if willing to participate. Inclusion criteria were patients who have been diagnosed as DM with nephropathy (characterized by uACR ≥ 30 mg/g Cr) and without nephropathy (characterized by uACR <30 mg/g Cr) based on the criteria of PERKENI 20152, while exclusion criteria included hemolysis, jaundice, lipemic examination, patients with a history of chronic liver disease (infection, malignancy), steroid drug use, cimetidine, trimethoprim and cephalosporin, poor nutrition, history of cardiocerebrovascular disease (heart and stroke), history of chronic renal failure.

The sample was taken by consecutive sampling method from the sample population that fulfilled the inclusion and exclusion criteria. The determination of sample size was based on an analysis of test difference of two unpaired averageand obtained a minimum sample amount that was 44 subjects.[11] The examination material in this research was the serum obtained from venous blood by using a tube without anticoagulant for determination of ADMA urine samples were taken for analysis of uACR.

Examination of ADMA levels using ELISA micro method with competitive principle.[12] Cut-off normal ADMA level was 0.66 µmol/L. Urine albumin and creatinine were each measured by a spectrophotometer and then calculated in the ratio (uACR). Microalbumin, used examination imunoturbidimetry method of the particles while creatinine used a Jaffe kinetic modification method.[13,14]

The data obtained were processed and analyzed univariable and bivariable. Data were presented regarding numbers and percentage for data of age and sex while for numerical data i.e. uACR value and ADMA serum level, mean and standard deviation (if normal distribution) or median and range (if abnormal data distribution) were used. Normality test of uACR value data, and ADMA level used the Shapiro-Wilk test. If the data were normally distributed then independent T-test analysis was used, whereas if data were not normally distributed Mann-Whitney test analysis was used. For the statistical testing, the significance level (significance) used was 5%.[11]

RESULTS AND DISCUSSION

While collecting the samples, 53 research subjects were obtained by using inclusion and exclusion criteria. Subjects were divided into two groups based on uACR value, diabetic nephropathic group was a group with uACR level ≥ 30 mg/g Cr (albuminuria) and non-diabetic nephropathic group was a group with uACR level < 30 mg/g Cr (non-albuminuria). The cut-off level was 0.66 µmol/L, determined based on a prior research conducted by Jayachandran et al., about diabetes mellitus patients from Asian race by using ADMA examination method, namely micro ELISA.[15] Table 1 below showed the research subject characteristics between the two groups.

The result of the normality test showed that the ADMA level did not have a normal distribution (p<0.05) so that the data will be presented in median and range form. The difference of ADMA serum level between the two groups can be seen in Table 2.

Table 1. Research subject characteristics

Characteristic	Group based on the uACR level	
	DN (albuminuria) (n=26) n (%)	Non DN (non-albuminuria) (n=27) n (%)
Age (years old)		
15-24	2 (7.7%)	0 (0%)
25-34	1 (3.8%)	1 (3.7%)
35-44	0 (0%)	3 (11.1%)
45-54	2 (7.7%)	3 (11.1%)
55-64	12 (46.2%)	12 (44.4%)
65-74	6 (23.1%)	7 (25.9%)
≥ 75	3 (11.5%)	1 (3.7%)
Gender		
Male	19 (73.1%)	13 (48.1%)
Female	7 (26.9%)	14 (51.9%)
ADMA serum level*		
<0.66 µmol/L	0 (0%)	22(81.5%)
≥ 0.66 µmol/L	26(100%)	5(18.5%)

Based on Table 2, it was showed that the median of ADMA level in DN group was increased as much as twice more than the non-DN group. The difference of ADMA level between the two groups was statistically significant with a p-value <0.05. in ADMA levels. Increased levels of ADMA is a marker of impaired renal function and have a role in proteinuria and decreased GFR through NO production disturbance mechanism.[8-10]

Table 2. The difference of ADMA serum level between nephropathy and non-nephropathy diabetic group

Variable	Group		P-value
	Non-DN (non-albuminuria) n=27	DN (albuminuria) n=26	
ADMA serum level			**<0.001***
Median	0.57	1.01	
Range (min - max)	(0.27-1.17)	(0.73-2.25)	

Note: comparative test was done by using Mann-Whitney test because the data were not normally distributed. Significant level based on p-value p <0.05. Sign of '*' showed that the p-value <0.05.

Table 3. The difference of ADMA level against subject characteristic

Variable	Normal ADMA level (n=22)	High ADMA level (n=31)	P-value
Body Mass Index (BMI)			0.872
Normal	13 (59.1%)	19 (61.3%)	
Overweight and obesity	9 (40.9%)	12 (38.7%)	
Dyslipidemia			0.143
Yes	13 (59.1%)	12 (38.7%)	
No	9 (40.9%)	19 (61.3%)	

Note: Analysis by using Chi-Square test

Based on Table 3, the analysis of research subject characteristic showed BMI variable and dyslipidemia. It consisted of 53 research subjects and divided into two groups based on ADMA level, namely normal ADMA level (<0.06 µmol/L) and high ADMA level (≥ 0.06 µmol/L).

The age group suffering from DM in this study was dominated by the 55-64 years old group, both for DN group and non-DN group. The result was similar with RISKESDAS study in 2013, stating that the prevalence of DM mostly was in the range of 55-64 years old.16 Increasing age factor is one of the risk factors for DM type 2 (DMT2) associated with decreased insulin secretion by β cell pancreas, and incidence of insulin resistance. In this study, male DM patients were more than females, similar with a epidemiology study result which suggested that the most DMT2 patients was in the > 40 years old age group with a prevalence rate of 30-40%.[17]

All the research subjects in DN group (100%) showed an increase in ADMA level, while the majority of subjects in the non-DN group showed a normal ADMA level (81.5%). This study results were similar to Jawalekar et al., study in India for DM patients.[18]

Diabetic nephropathy disease is characterized by prolonged hyperglycemia, morphological changes and decreased renal function. The state of hyperglycemia stimulates the advanced glycation end-product (AGE) pathway, activation of protein kinase C (PKC), elevated hexosamine pathways and increased production of proinflammatory cytokines such as TNF-α is capable to damage the endothelial such as in glomerular endothelium. Activation of all these pathways result in increased formation of free radicals (ROS). These free radicals can increase the activity of protein arginine methyltransferases (PRMTs) that help the process of L-arginine protein methylation into ADMA and decrease the activity of DDAH (dimethylarginine dimethylaminohydrolase) enzyme to degrade ADMA resulting in intracellular ADMA accumulation. Asymmetric dimethylarginine is an amino acid homologous of endogenous L-arginine that undergoes methylation during protein turnover process and is continously produced in normal protein metabolism conditions as L-Arginine residue. After the process of proteolysis, ADMA compounds will be found in the cytoplasm and can be detected in the blood circulation. Increased ADMA levels indicate endothelial dysfunction in DN. The more severe the kidney damage higher the value of Uacr and ADMA levels.[19-21]

In patients with DM an increased of ADMA levels can occur but in this study, the majority of subjects in the DM group of non-DN (81.5%) had normal ADMA levels. Normal ADMA levels can be caused by the use of antioxidant drugs, estrogen, aspirin and vitamin A but the mechanism is unclear.[19] In this study, 5 subjects in the non-DN group (18.5%) experienced elevated ADMA levels. Increased ADMA levels in these five subjects were due to a history of hypertension. Hypertension leads to vasoconstriction, increased peripheral resistance and free radicals that have an impact on decreasing the DDAH activity to degrade ADMA. According to Anderson et al., ADMA levels in patients with DMT2 may also increase acutely when eating foods in high fat and sugar before taking blood samples.[22] The limitation of obtaining of food information consumed by research subjects made the research more difficult.

Based on Table 2, showed that the median value of ADMA levels increased almost twice more in the DN group compared to the non-DN group. The results of this study were similar to the results of previous studies conducted by Tarnow et al., in diabetic nephropathy patients.[19]

This study looked at the effect of BMI and dyslipidemia on the increasing of ADMA level. Table 3 showed that the BMI and dyslipidemia variables did not affect elevated ADMA levels with a p-value >0.05. The result of this study was similar to the results of Hariawan's research in 2008 who suggested that BMI, long-suffering DM and the presence or absence of dyslipidemia did not affect the elevated ADMA level (p-value > 0.05) in 80 DMT2 and normal subjects.[23]

Most of the study subjects had a normal BMI, and showed no difference in elevated ADMA levels between the two groups. Body mass index values did not measure fat directly but assessed the nutritional status first, particularly related to deficiency and overweight/obesity.24 Obesity is associated with elevated levels of ADMA and obesity assessment is not enough simply by assessing BMI, other anthropometric variables such as arm circumference, waist and pelvis are included[10] In this study most of the study subjects had a normal BMI and did not differ between two groups (p > 0.05). The results of this study were similar to those by Hanai.[10]

Most of the study subjects in the group of normal ADMA level in this study experienced dyslipidemia (59.1%). Dyslipidemia was one of the risk factors for endothelial

dysfunction. Increased methyltransferase activity by high LDL was considered to contribute in elevating ADMA level due to dyslipidemia.[15] However, in the group of high ADMA level, the majority of the study subjects (61.3%) did not experience dyslipidemia and did not affect the increase of ADMA level. The results of this study were similar to Ozdogan et al., study who showed no difference between cholesterol, triglyceride, HDL, and LDL variables with ADMA level ($p > 0.05$).25 Normal levels of cholesterol, and triglycerides in the group of high ADMA level can be caused by anti-cholesterolemia/anti-triglyceridemic therapy as well as a good diet. This study had the limitation such as less stringent selection criteria and the absence of complete data on compliance in DM patients (educational background that affects the understanding of DM disease which is the silent disease and their risk factors).

All subjects in the diabetic nephropathy group (100%) showed an elevated serum ADMA level compared to the diabetic non-nephropathy group (85.2%). In the non-DN group there were 5 subjects (18.5%) who had elevated ADMA levels. Once traced further, the five subjects had hypertension. Hypertension plays a major role as the cause and progression of DN and is a marker of macro vascular, and micro vascular complications in patients with DM. Based on the definition of ADA 2017 diabetic nephropathy is characterized by the presence of hypertension, albuminuria and/ or decreased GFR.26 Hypertension in diabetic is a comorbid disease that can affect ADMA levels. According to Hariawan[23] who studied 80 subjects of elderly who experienced DMT2 hypertension had a correlation to elevated levels of ADMA.23 In addition to hypertension in the study, the use of antioxidant drugs, estrogen, aspirin, and vitamin A was also estimated to affect ADMA levels but the mechanism was still unclear.[19]

CONCLUSIONS AND SUGESSTION

Asymmetric Dimethylarginine (ADMA) serum levels in the DM group with DN increased significantly compared with non-DN group. The severity of albuminuria in DN affected ADMA serum levels. So, ADMA serum levels could be considered as additional tests to determine the presence of diabetic nephropathy in DM patients besides other laboratory tests like albuminuria or uACR.

Further research is needed to observe the role of ADMA in helping to establish the diagnosis of diabetic nephropathy.

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