ADMA as a Marker of Endothelial Dysfunction in Prediabetic Women

Fatimah Eliana, Pradana Suwondo, Lukman H. Makmun, Dante S. Harbuwono

Department of Internal Medicine, Faculty of Medicine,
University of Indonesia - Cipto Mangunkusumo Hospital. Jl. Diponegoro no. 71, Jakarta, Indonesia.

Correspondence mail to: *fat_eliana_sppd@yahoo.co.id*

ABSTRACT

Aim: *to determine the correlation between glycohemoglobin (HbA1c) with level of asymmetric dimethylarginine (ADMA) in serum, between HbA1c and value of brachial artey flow-mediated dilatation (FMD) by ultrasound study, and between ADMA serum and FMD in prediabetes women.*

Methods: *the study was done in prediabetes women aged between 30-55 years of age in Cipto Mangunkusumo Hospital Jakarta (RSCM). Prediabetes was based on PERKENI criteria. Subject with fasting blood glucose less than 126 mg/dL and 2-hours blood glucose less than 200 mg/dL met the criteria. Laboratory test of HbA1c and ADMA plasma were performed. To asses brachial FMD, the left brachial artery diameter was measured both at rest and during reactive hyperemia. Increased flow was induced by inflation of pneumatic tourniquet around the forearm to a pressure of 50 mm Hg upper systolic pressure for 5 minutes, followed by release. Measurements of arterial diameter were performed at end diastole at rest and 60 seconds after cuff release. The vessel diameter in scans after reactive hyperemia was expressed as the percentage relative to resting scan (100%). All ultrasound scans were analyzed by a single reader.*

Results: *from 41 prediabetes subjects could be found correlation between ADMA serum with 2-hours post prandial blood glucose (p 0,003 and r 0,457) and HbA1c (p <0,001 and r 0,720). We also found correlation between FMD value with 2-hours post prandial blood glucose (p 0,01 and r -0.487) and HbA1c (p <0,001 and r -0.763). Besides that, there was correlation between ADMA serum with FMD value (p <0,001 and r -0,617). From multivariate analysis, we could determine that HbA1c is the influential factor of ADMA serum and FMD.*

Conclusion: *in prediabetes women there was correlation between HbA1c with ADMA, between HbA1c with FMD and between ADMA serum with FMD.*

Key words: *prediabetes, HbA1c, ADMA, FMD.*

INTRODUCTION

Several epidemiologic studies have established evidence on the correlation between glycemic status and the risk of cardiovascular diseases. Atherosclerosis Risk in Communities (ARIC) study in 2010 has also reported that cardiovascular defect has already existed in subjects who do not have diabetes and mortality increases when there is an increase in glycohemoglobin (HbA1c) more than 5.5%.[1]

In women, it is a cause of more concern since the *Diabetes Epidemiology Collaborative analysis of Diagnostic criteria in Europe* (DECODE) study has established evidence that although risk of death due to cardiovascular disorder in diabetic men is higher compared to diabetic women; however, the risk of cardiovascular defect in pre-diabetic women is higher than pre-diabetic men with a hazard ratio 2.07 compared to 1.86.[2] *Guidelines* on diabetes, *pre-diabetes*, and cardiovascular diseases in 2007 has suggested that glucometabolic change is a risk factor of cardiovascular defect and mortality in women and it is included in class IIa category with level of evidence B, which means that it is useful supported by evidence and opinion.[3]

Endothelial dysfunction is the begining of atherosclerosis process that may result in cardiovascular defect.[4-8] In endothelial dysfunction, there is an increase of asymmetric dimethylarginine (ADMA) level, which will block NO synthase (NOS) in the endothelial cells leading to a decrease in NO synthesis.[9-10] Now, ADMA has been approved as a novel cardiovascular risk marker.[11-13] Several studies had demonstrated evidences on increased ADMA level in diabetes patients.[14-15]

Flow Mediated Dilatation (FMD) examination has long been used as an indicator of endothelial dysfunction.[16-18] Arteries that suffer from atherosclerosis

had already experienced endothelial dysfunction to produce NO resulting in a suboptimal dilatation of the arteries. [19-23]

The aim of our study was to recognize the correlation between HbA1c and endothelial function in pre-diabetic women, i.e. by examining ADMA serum level and FMD to assess the altered post-occlusion brachial artery diameter. Another aim was to recognize the correlation between ADMA level, as an indicator of NO synthesis, and FMD level as an indicator of endothelial dysfunction.

Little has been studied on pre-diabetic subjects, particularly women. In Indonesia, there has been no study on correlation between HbA1c level and ADMA serum level with FMD, either in diabetic patients, normal people or pre-diabetics.

Data obtained from this study may be useful to explain whether there has been already an endothelial dysfunction in pre diabetic women and assuming on how far the dysfunction had occurred in keeping with the increase of HbA1c level; therefore, it is expected that if care had been taken to decrease HbA1c level, then endothelial function would return to normal.

METHODS

The design of our study was cross-sectional. Study subjects were female workers at Cipto Mangunkusumo Hospital, age 30-55 years old who had fulfilled the criteria of pre-diabetic and had signed informed consent forms. The rational for selecting these subjects was based on RISKERDAS 2009 and pre-diabetic epidemiologic data in Depok by Yunir et al (2009), most prediabetic subjects were 30-55 years old.[24-25]

Subjects were excluded if they had a disease or in conditions which could affect the result of HbA1c, ADMA and FMD examination, such as pregnancy or lactation, acute infection or in hospitalization, anemia, menopause, diabetes or hypertension, cardiovascular disease, chronic renal or liver dysfunction, smoking, and consuming corticosteroids, estrogen, adrenergic beta-receptor agonist, nitrate or other vasodilator agents.[10,22] Estimation of sample size was calculated by using the correlation formulation by r 0.5. Based on the calculation, minimal subjects needed was 38 subjects.[26]

The diagnosis of pre-diabetes, impaired fasting glucose (IFG), and/or impaired glucose tolerance (IGT) were established based on the results of oral glucose tolerance test (OGTT). IFG was established when there was blood glucose level after 10-12 hour fasting of 100-125 mg/dL. Diagnosis of IGT was established if the 2-hour blood glucose level after 7 5 gram glucose loading of 140-199 mg/dL.[27-28]

HbA1c examination was performed by using High *Performance Liquid Chromatography* (HPLC) method according to American Diabetes Association standard.[29] The examination of ADMA level was performed by using ELISA method with a normal range of 0.4-0.75 µmol/L (80-150 ng/mL).[30] FMD examination was conducted in a room temperature between 20-25oC by using 7.0 MHz ultrasonography transducer. The measured artery was brachial artery, located 2-15 cm above the elbow which was scanned longitudinally. The subject rested in supine position for at least 5 minutes before the first scan (basal diameter) was recorded. The rubber cuff was placed around the lower arm and the air was pumped until the pressure was 50 mmHg above systolic blood pressure for 3-5 minutes. The second scan was taken 60-120 second after the rubber cuff had been deflated (hyperemia diameter). The measurement of arterial diameter was performed by using ultrasonography caliper, i.e. the distance between the "m" line, which was the edge between tunica media and anterior adventitia at the end diastole in accordance with R wave in ECG. FMD examination was expressed as: [(hyperemia diameter – basal diameter) / basal diameter] x 100%.[23]

The obtained data were processed with SPSS 16, and presented in the form of distribution and correlation tables. The result of the study was written based on the normality of data distribution. Data with normal distribution presented as mean (average) value and standard deviation (SD). While for data with abnormal distribution were presented with median value, 25th and 75th percentile.

Bivariate correlation between independent variables, i.e. age, body mass index, fasting blood glucose, 2-hour post prandial blood glucose level, HbA1c, total cholesterol, LDL, HDL and triglyceride and dependent variables including ADMA level and FMD level, was processed with Pearson test when the data were normally distributed or with Spearman test if it did not meet the normality of data. When there was more than one significant factor, a multivariate analysis was conducted with linear regression method. Variables included in multivariate analysis were those with $p < 0.25$ and $r < 0.8$ in bivariate analysis. The significance level used in this study was 5%.

Our study had been approved by ethical committee of Faculty of Medicine, University of Indonesia and had received permission from the director of Cipto Mangunkusumo Hospital. The limitation of our study was due to its cross sectional design, which could not

provide evidence for the causative association of fasting blood glucose level, the 2-hour blood glucose level after 75 g of glucose intake, HbA1c, body mass index, and lipid profile with ADMA serum level and FMD. Such design may only explain correlative association between those variables.

RESULTS

Our study was conducted at Cipto Mangunkusumo Hospital between August and October 2010, and we found 41 subjects of pre-diabetic women who fulfilled the inclusion criteria.

Table 1. General subject characteristics

Characteristics	Mean ± SD Median (25^{th};75^{th} percentile)
Age (years)	42.98 ± 5.88
Body Mass Index (kg/m^2)	29.11 ± 4.33
Abdominal ciricumference (cm)	87.29 ± 9.99
Fasting blood glucose (mg/dL)	93.51 ± 11.03
2-hour blood glucose OGTT (mg/dL)	157.54 ± 11.47
HbA1c (%)	5.98 ± 0.29
Total choleseterol (mg/dL)	203.98 ± 42.74
LDL cholesterol (mg/dL)	136.12 ± 33.49
HDL cholesterol (mg/dL)	47.15 ± 8.002
Triglycerides (mg/dL)	151 ± 134.5;190.5
ADMA (µmol/L)	0.653 ± 0.131
FMD (%)	11.09 ± 2.68

The mean body mass index of the subjects was 29.11 (4.33) kg/m^2. It was consistent with the obesity classification, therefore, 19 subjects (46.3%) were included in grade 2 obesity, 16 subjects (39%) were in grade 1 category and the other three subjects (7.3%) were in overweight classification. There were 3 subjects (7.3%) who were classified as normal weight subjects and none was classified in underweight. It demonstrated that 92.7% pre diabetic subjects in our study were in overweight classification. Such condition was in accordance with pre-diabetes consensus issued by *American College of Endocrinology/American Association of Clinical Endocrinologist* (ACE/AACE) 2008, which then being reconfirmed through the pre-diabetes consensus by Indonesian Diabetes Experts Association or *Persatuan Ahli Diabetes Indonesia* (Persadia) in 2010 that one of the risk factors of pre diabetes is overweight or obesity. The condition was associated with insulin resistance in those subject.[29,31]

The mean abdominal circumference of the subjects was 87.29 (9.99) cm. Based on the obesity criteria for Asia Pacific population in 2000 with a limit of abdominal circumference more than 80 cm in women, then we would have 33 subjects (80.5%) classified as obese.[32]

Based on pre-diabetes criteria, the subjects in our study may be classified into 3 groups, i.e. 2 subjects (4.9%) in impaired fasting glucose group, 32 subjects (78%) with impaired glucose tolerance and 7 subjects (17.1%) were included in the combined group of IFG and IGT. Such condition is consistent with the result of pre diabetic epidemiologic study conducted in Depok, West Java by Yunir in 2009 which showed that of 329 pre-diabetic subjects, 12.15% were included in IFG group, 71.12% were in IGT group and 16.71% were classified in the combined IFT and IGT group.[25] The mean value of HbA1c level in the pre-diabetic subjects of our study was 5.98 % (0.29), which was consistent with the pre-diabetic criteria issued by the *American Diabetes Association* in 2010, i.e. 5.7-6.4%.[29]

Based on the criteria of metabolic syndrome from *International Diabetes Federation including* HDL less than 50 mg/dL and triglyceride more than 150 mg/dL, which were used as some indicators of metabolic syndrome, our study showed that 27 subjects (65.9%) had HDL less than 50 mg/dL and 35 subjects (85.4%) had triglyceride level more than 150 mg/dL.[32]

Correlation Between Independent Variables and ADMA Level with FMD Value

There was a significant correlation between ADMA levels and blood glucose after 75 g glucose intake (post-prandial blood glucose level), with p value of 0.003 and correlation 0.457. There was significant correlation between ADMA level and HbA1c, $p < 0.001$ and correlation 0.720 as well as significant correlation between FMD level and post-prandial blood glucose level with $p < 0.001$ and correlation -0.487, and with HbA1c level $p < 0.001$ and $r = -0.763$.

Subsequently, a multivariate analysis was performed for numeric variables with $p<0.25$ and $r<0.8$ against ADMA level including post-prandial blood glucose level, HbA1c and total cholesterol. The result of linear regression test showed that only HbA1c had a correlation with ADMA and by knowing the level of HbA1c, we could predict serum ADMA level. Logistic regression multivariate analysis also showed an odds ratio of HbA1c against ADMA level of 12.22 (95% CI, 2.531-59.021). The probability of pre-diabetic subjects with HbA1c > 6% to have abnormal ADMA level, i.e. more than 0.75 µmol/L, was 92.44%.

Multivariate analysis of numeric variable with $p < 0.25$ and $r < 0.8$ against FMD had also been performed.

Table 2. Correlation between independent variables and ADMA level with FMD value

Variables	ADMA		FMD	
	p	r	p	r
Age (year)	0.979	0.004	0.281	-0.172
Body mass index (kg/m^2)	0.725	0.057	0.873	-0.026
Abdominal circumference (cm)	0.671	-0.068	0.416	-0.130
Fasting blood glucose (mg/dL)	0.547	0.097	0.708	-0.060
2-hour OGTT blood glucose (mg/dL)	0.003	0.457	0.001	-0.487
HbA1c (%)	<0.001	0.720	<0.001	-0.763
Total cholesterol (mg/dL)	0.233	0.190	0.497	-0.109
LDL cholesterol (mg/dL)	0.738	0.054	0.571	-0.091
HDL cholesterol (mg/dL)	0.483	0.113	0.539	-0.099
Triglycerides (mg/dL)	0.353	0.149	0.552	-0.096

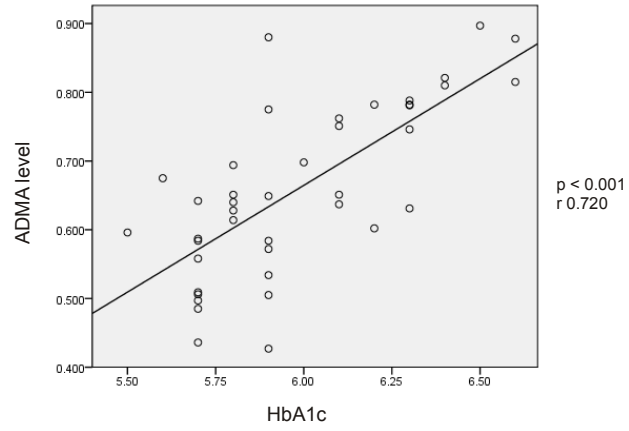

Figure 1. Correlation of linear regression between HbA1c and ADMA

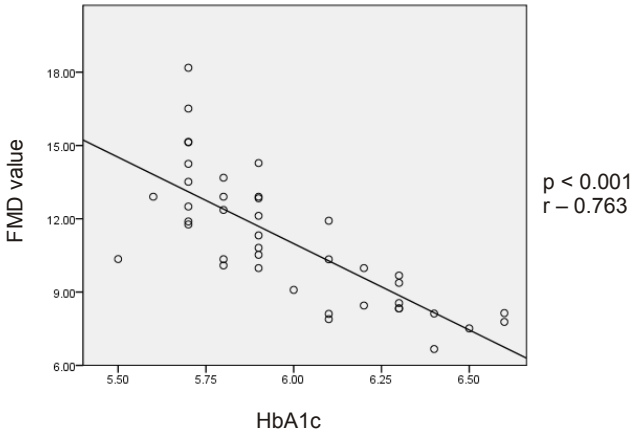

Figure 2. Correlation of linear regression between HbA1c and FMD

The variables were post-prandial blood glucose level and HbA1c. Linear regression test also showed that only HbA1c level had a correlation with FMD and by knowing the level of HbA1c, we could predict FMD value. Logistic regression has also showed HbA1c

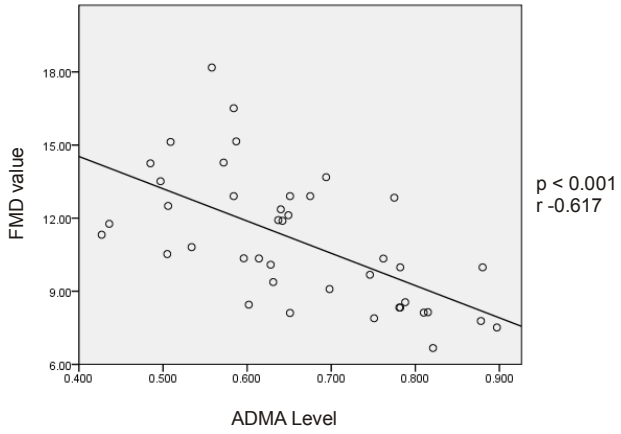

Figure 3. Correlation of linear regression between ADMA and FMD

against FMD with odds ratio of 38.57 (95% CI, 4.255-349.672). The probability of pre-diabetic subjects with HbA1c > 6% to have an abnormal FMD, i.e. less than 11%, was 97.47%.

The result of correlation statistic analysis between FMD and ADMA level indicated that there was a significant correlation and strong association, with $p < 0.001$ and $r = -0.617$.

DISCUSSION

Until now, there has been no study report about the correlation between ADMA and HbA1c level in pre-diabetic subjects; however, there are several reports in subjects with Diabetes Mellitus by Hannu Paiva in 2003, Yan Xiong in 2004 and Nakhjavani in 2010.[33-36] A study by Yan Xiong in 2004 in China also provided evidence that increased ADMA level had no correlation with the duration of illness of diabetes melitus, but rather correlates with metabolic control.[34]

As many as 28 pre-diabetic subjects participated in our study (68.3%) have normal ADMA level, with mean value of 0.653 µmol/L (0.131). A study by Dildar Konukoglu (2007) in 25 pre-diabetic subjects also found a normal average ADMA level which was 0.47 µmol/L (0.13).38 Another study by Anderson (2007) in 239 pre-diabetic subjects found ADMA level at a little above normal value, i.e. 0.76 µmol/L (0,43;1,93).[38]

Several studies had established evidence that ADMA level is an independent risk factor of cardiovascular defect. ADMA level is an analog of endogenous L-arginine which affects NO metabolism by inhibiting NOS activity. Wang in 2009 compared ADMA level in 608 patients with coronary heart disease (CHD) and 402 patients without CHD. The study showed that ADMA level in patients with CHD was higher than patients without CHD, with p <0.003 and odds ratio 1.32. The risk of having heart attack (hazard ratio) for 3 years also inkeeping with increased ADMA level, i.e. 3.2.[33]

A cohort study, *Atherosclerosis Risk in Communities* (ARIC) showed that the higher the level of HbA1c, the higher also the incidence of cardiovascular disorder and mortality. Such a level of HbA1c even had higher correlation compared to fasting blood glucose level. Every increase of HbA1c will result in increased risk of coronary heart disease (hazard ratio 1.5), ischemic stroke (hazard ratio 1.55), and death (hazard ratio 1.8).[1]

Our study in pre-diabetic subjects demonstrated that there is a positive correlation and association between ADMA level and HbA1c. The higher the level of HbA1c in pre-diabetic subjects, the higher the level of ADMA, which means worse metabolic control leading to greater risks of endothelial dysfunction and vasculopathy. The probability of pre-diabetic subjects with HbA1c level was more than 6% to have ADMA level more than 0.75 µmol/L was 92.44%.[33,39]

In our study, there is no evidence of correlation between ADMA level with LDL cholesterol and triglyceride level. It may occur since most subjects in our study (92.68%) had normal cholesterol and did not have prior hypercholesterolemia. Vladimirova Kitova, 2008 in Bulgary had different results, he found a significant correlation between ADMA level with total cholesterol (p<0.001 and r = 0.603). It may happen due to severe hypercholesterolemia condition of the subjects in that study with a mean value of total cholesterol was 336 mg/d.[40]

Reduced FMD value showed that there is endothelial dysfunction. The result of our study in pre-diabetic subjects also suggested association and negative correlation between FMD and HbA1c level. The probability of pre-diabetic subjects with HbA1c > 6% to have FMD less than 11% was 97.47%. Such condition indicates that there is a disturbance of endothelial dilatation function in pre-diabetic patients as HbA1c level elevates.

A study by Ying Su in diabetic subjects found a significant FMD value difference between diabetic group (5.27%) and normal group (10.68%). While in 30 pre-diabetic subjects, a study found FMD level of 9.87%.[41] FMD examinations in 41 pre-diabetic subjects demonstrated a higher average, 11.09% (2.68).

Our study does not provide evidence on correlation between FMD value with LDL cholesterol and triglyceride level. A case control study by Maciej (2009) in Poland with male subjects aged less than 45 years old also demonstrated similar result, that is FMD value correlated with diabetes but had no association with body mass index, hypercholesterolemia, hypertension or smoking. Maciej study concluded that FMD examinations could identify subjects with high CHD risk.[42] A study by Park Hyun Woong in Korea reported in 2010 also did not find any correlation between FMD with total cholesterol (p 0.016 and r 0.012), LDL cholesterol (p 0.016 and r 0.16), HDL cholesterol (p 0.5 and r -0.1) and triglyceride (p 0.61 and r 0.014).[43]

Correlation statistical analysis between FMD and ADMA level was performed with Pearson test as the data showed normal distribution. There is an association and strong correlation between FMD and ADMA level, p < 0.001 and correlation = -0.617. A study by Juonala, 2007 in male subjects aged 24-39 years old in Finland also found a correlation between ADMA and FMD level with p 0.003.[41] Vladimirova Kitova, 2008 reported a significant correlation between FMD and ADMA level with p<0.001.[41] This demonstrates that both ADMA and FMD examination can be utilized to assess endothelial dysfunction in pre-diabetic women.

The limitation of this study is that cross sectional design could not be used to prove causative correlation between fasting blood glucose level, 2-hour blood glucose level after 75g glucose intake, HbA1c, body mass index, and lipid profile with plasma ADMA level and FMD. Such a design may only explain correlative relation between those variables. Moreover, there are many other factors that have not been evaluated in our study such as the difference in physical activity, insulin level, Homeostasis Model Assesment Insulin Resistance (HOMA IR); therefore, the result of our study could not be generalized to other population.

CONCLUSION

Based on the study results, we could conclude that there is a correlation between glycohemoglobin (HbA1c) and endothelial function in pre-diabetic women as proven by:

a. A significant association with a strong correlation between HbA1c with ADMA level. The higher HbA1c level, the higher ADMA level.
b. A reverse significant association with strong correlation between HbA1c level with FMD level. The higher HbA1c level, the lower FMD value goes.
c. A reverse significant association with strong correlation between ADMA level with FMD value. The higher ADMA level, the lower FMD value.

Endothelial dysfunction in pre-diabetic women has already taken place when HbA1c level is more than 6%. Therefore, intervention such as lifestyle change must be taken and if necessary, pharmacotherapy is administered to decrease HbA1c level to less than 6% to prevent vascular changes.

REFERENCES

1. Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med. 2010;362:800-11.
2. Gender difference in all-cause and cardiovascular mortality related to hyperglycemia and newly-diagnosed diabetes. The Decode Study Group. Diabetologia. 2003;46:608-17
3. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases. European Society of Cardiology (ESC). 2007.
4. Landim MBP, Filho AC, Chagas ACP. Asymmetric dimethylarginine (ADMA) and endothelial dysfunction: implications for atherogenesis. Clinics. 2009;64(5):471-8.
5. Verma S, Buchanan MR, Anderson TJ. Endothelial function testing as a biomarker of vascular disease. Circulation. 2003;108:2054-9.
6. De Vriese AS, Verbeuren TJ, Van der Voorde J, Lameire NH, Vanhoutte PM. Endothelial dysfunction in diabetes. Br J Pharmacol. 2000;130(5):963-7.
7. Maiese K. Shang YC. Chong ZZ. Hou J. Diabetes mellitus: channeling care through cellular discovery. Curr Neurovasc Res. 2010;7(1):59-64.
8. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, et al. Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. Circulation. 1993;8:2149-55.
9. Kocaman SA. Asymmetric dimethylarginine, NO and collateral growth. Ana do lu Kar di yol Derg. 2009;9:417-20.
10. Cooke JP. Does ADMA cause endothelial dysfunction? Biol. 2000;20:203.
11. Stuhlinger MC, Oka RK, Graf EE, Kimoto M, Balint RF, Cooke JP. Endothelial dysfunction induced by hyperhomocysteinemia: role of ADMA. Circulation. 2003;108:933-8.
12. Jellinger PS. Metabolic consequences of hyperglycemia and insulin resistance. Clinical Cornerstone. 2007;8:S30–S42.
13. Anderssohn M, Schwedhelm E, Lüneburg, Ramachandran S. Vasan RS, Böger RH. Asymmetric dimethylarginine as a mediator of vascular dysfunction and a marker of cardiovascular disease and mortality: an intriguing interaction with diabetes mellitus. Diab and Vasc Dis Res. 2010:7;105-18.
14. Hashimoto M, Miyamoto Y, Matsuda Y, Akita H. New methods to evaluate endothelial function: non-invasive method of evaluating endothelial function in humans. J Pharmacol Sci. 2003;93:405-8.
15. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, Tsuji H, Reaven GM, Cooke JP. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. Circulation. 2002;106;987-92.
16. Peretz A, Leotta DF, Sullivan JH, Trenga CA, Sands FN, Aulet MR, et al. Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. BMC Cardiovascular Disorder. 2007;7:11.
17. Witte DR, Westerink J, de Koning EJ, van der Graaf Y, Grobbee DE, Bots ML. Is the Association between flow-mediated dilation and cardiovascular risk limited to low-risk populations? J Am Coll Cardiol. 2005;45:1987-93.
18. Lieberman EH, Gerhard MD, Uehata A, Selwyn AP, Ganz P, Yeung AC, et al. Flow induced vasodilatation of the human brachial artery is impaired in patient less than 40 years of age with coronary artery disease. Am J Cardiol. 1996;78:1210-4.
19. Roman MJ, Naqvi TZ, Gardin JM, Gerhard-Herman M, Jaff M, Mohler E. Clinical application of noninvasive vascular ultrasound in cardiovascular risk stratification: a report from the American Society of Echocardiography and the Society of Vascular Medicine and Biology. J Am Soc Echocardiogr. 2006;19:943-54.
20. Neunteufl T, Katzenschlager R, Hassan A, Klaar U, Schwarzacher S, Glogar D, et al. Systemic endothelial dysfunction is related to the extent and severity of coronary artery disease. Atherosclerosis. 1997;129:111-8.
21. Anderson TJ, Uehata A, Gerhard MD, Meredith IT, Knab S, Delagrange D, et al. Close relation of endothelial function in the human coronary and peripheral circulations. J Am Coll Cardiol. 1995;26:1235-41.
22. Kelm M. Flow-mediated dilatation in human circulation: diagnostic and therapeutic aspects. Am J Physiol Heart Circ Physiol. 2002;282:1-5.
23. Corretti CM, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery. JACC. 2002;39:257-65.
24. Mihardja L, Delima, Siswoyo H, Ghani L, Soegondo S. Prevalence and determinants of diabetes mellitus and impaired glucose tolerance in Indonesia (a part of basic health research/Riskesdas). Acta Med Indones-Indones J Intern Med. 2009;41(4):181-5.
25. Yunir E, Waspadji S, Rahajeng E. The pre-diabetic epidemiological study in Depok, West Java. Acta Med Indones-Indones. J Intern Med. 2009;41(4):170-4.
26. Madiyono B, Moeslichan S, Sastroasmoro S, Budiman I, Purwanto SH. Perkiraan besar sampel. Dalam: Sastroasmoro S, Ismael S, ed. Dasar-dasar metodologi penelitian klinis edisi ke-3. 2008:302-331.
27. Nathan DN, Davidson MB, DeFronzo R, Heine RJ, Henry, RH, Pratley R, et al. Impaired fasting glucose and impaired glucose tolerance: implications for care. Diabetes Care. 2007;30(3):753-9.

28. Garber AJ, Handelsman Y, Einhorn D, Bergman DA, Bloomgarden ZT et al. Diagnosis and management of prediabetes in the continuum of hyperglycemia. When do the risks of diabetes begin? A consensus statement from the American College of Endocrinology and The American Association of Clinical Endocrinologist. Endocrine Practice. 2008;14:933-48.
29. Standards of medical care in diabetes 2010. American Diabetes Association. Diabetes Care. 2010;33:S11-61.
30. Miyazaki H, Matsuoka H, Cooke JP, Usui M, Ueda S, Okuda S, et al. Endogenous nitric oxide synthase inhibitor: a novel marker of atherosclerosis. Circulation. 1999;99:1141-6.
31. Soeatmadji DW, Pranoto A, Manaf A, Pandelaki K, Suastika K, Soegondo S, et al. Buku panduan pengelolaan dan pencegahan prediabetes. PB PERSADIA. 2009:4-8.
32. Inoue S, Zimmet P. The Asia-Pacific perspective: redefining obesity and its treatment. International association for the study of obesity (IASO). International obesity taskforce. WHO Western Asia Pacific region. 2000:17-20.
33. Konukoglu D, Firtina S, Serin O.The relationship between plasma asymmetrical dimethyl-L-arginine and inflammation and adhesion molecule levels in subjects with normal, impaired, and diabetic glucose tolerance. Metabolism Clinical and Experimental. 2008;57:110-5.
34. Paiva H, Lehtima T, Laakso J, Ruokonen I, Rantalaiho V, Worte O, et al. Plasma concentrations of asymmetric-dimethyl-arginine in Type 2 Diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy. Metabolism. 2003;52:303-7.
35. Yan Xiong, Minxiang Lei, Sihai Fu, Yunfeng Fu. Effect of diabetic duration on serum concentrations of endogenous inhibitor of nitric oxide synthase in patients and rats with diabetes. Life Sciences. 2005;77:149–59.
36. Nakhjavani M, Karimi-Jafari H, Esteghamati A, Khalilzadeh O, Asgarani F, Ghadiri-Anari A. ADMA is a correlate of insulin resistance in early-stage diabetes independent of hs-CRP and body adiposity. Ann Endocrinol Paris. 2010.
37. Juonala M, Viikari JSA, Alfthan G, Marniemi J, Kähönen K, Taittonen L, Laitinen T, Raitakari OT. Brachial artery flow-mediated dilation and asymmetrical dimethylarginine in the cardiovascular risk in young Finns study. Circulation. 2007;116:1367-73.
38. Anderson JL, Carlquist JF, Roberts WL, Horne BD, May HT, Schwarz EL, et al. Asymmetric dimethylarginine, cortisol/cortisone ratio, and C-peptide: Markers for diabetes and cardiovascular risk? American Heart J. 2007;153:67-73.
39. Wang Z, Tang W, Cho L, Brennan DM, Hazen SL. Targeted metabolomic evaluation of arginine methylation and cardiovascular risks. Potential mechanisms beyond nitric oxide synthase Inhibition. Arterioscler Thromb Vasc Biol. 2009;29:1383-91.
40. Vladimirova-Kitova L, Deneva T, Angelova E, Nikolov F, Marinov B, Mateva N. Relationship of asymmetric dimethylarginine with flow mediated dilatation in subjects with newly detected severe hypercholesterolemia.Clin Physiol Funct Imaging. 2008;28:417–25.
41. Ying S, Xiao-Min L, Yang-Ming S, Yue-Ying W, Ying L, Yun W. Endothelial dysfunction in Impaired Fasting Glycemia, Impaired Glucose Tolerance, and Type 2 Diabetes Mellitus. Am J Cardiol. 2008;102:497–8.
42. Kaźmierski M, Michalewska-Włudarczyk A, Krzych LJ, Michał Tendera M. Diagnostic value of flow mediated dilatation measurement for coronary artery lesions in men under 45 years of age. Cardiology Journal. 2010;17(3):288–92.
43. Hyun-Woong Park, Taek Geun Kown, Ki-Young Kim, Jang-Ho Bae. Diabetes, insulin resistance and atherosclerosis surrogates in patients with coronary atherosclerosis. Korean Circ J. 2010;40:62-7.