Association of Metabolic Syndrome with Albuminuria in Diabetes Mellitus Type 2

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ABSTRACT

Introduction: Metabolic syndrome is a risk factor for cardiovascular disease as well as the occurrence of chronic kidney disease. According to IDF, metabolic syndrome is diagnosed when central obesity has two or more metabolic abnormalities. Several previous studies reported an significant association found between the metabolic syndrome with albuminuria. In Indonesia, association of metabolic syndrome with albuminuria in type 2 diabetes have not been reported yet. The aims of this study was to analyze the association of metabolic syndrome with albuminuria in type 2 diabetes patients.

Methods: This was an analytic observational study with cross-sectional design. 131 diabetes mellitus type 2 patients were taken as sample. Data was analyzed using Spearman Correlation and Mann-Whitney test. Significance level used was 0.05.

Results: From 131 subjects with metabolic syndrome, normoalbuminuria proportion was 65.4%, microalbuminuria 27.1%, and macroalbuminuria 7.5%. There was significant association between systolic blood pressure with albuminuria, p=0.000, r=0.325. Fasting blood glucose with albuminuria, p=0.01, r=0.223, but no significant association between diastolic blood pressure with albuminuria was found with p=0.153, r=0.125, waist circumference with albuminuria, p=0.311, r=0.089, low HDL with albuminuria p=0.771, r=-0.025. Hypertriglyceridemia with albuminuria, p=0.727 and r=0,031.

Conclusion: The results indicate a strong association between the components of metabolic syndrome especially systolic blood pressure and fasting blood glucose with albuminuria. Whereas in diastolic blood pressure, waist circumference, low HDL, and hypertriglyceridemia no significant associations found

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Introduction

Metabolic syndrome is a major health problem nowadays. International Diabetes Federation (IDF) believes that groups of these factors influence the global epidemic of diabetes mellitus type 2 (DMT2) and cardiovascular disease, the syndrome consists of several risk factors including visceral obesity, dyslipidemia, insulin resistance, impaired glucose tolerance, and increased blood pressure.¹ The disease is related to lifestyle such as physical inactivity, junk food, carbohydrates, and smoking.

Metabolic syndrome affects about 40% of the population above 50 years old in United States and almost 30% in Europe. Tjokroprawiro reported that the prevalence of metabolic syndrome in a population over 40 years old undergoing a medical examination in Surabaya ranged from 32%. In addition, it was reported 43.3% were treated in DMT2 and 59.0% were treated in naïve DMT2 as observed in private clinics, while

*Correspondence: drthamrin1@gmail.com © 2019 Biomolecular and Health Science Journal. All rights reserved Available at https://e-journal.unair.ac.id/BHSJ the prevalence of metabolic syndrome in people with DMT2 with obesity was 81.7%.²

Metabolic syndrome is closely related to cardiovascular disease (CVD). In addition, recent studies show an association between metabolic syndrome and the risk of chronic kidney disease (CKD) and microalbuminuria.³ Study of Hao, et al. (2006) explained that there was a significant correlation between metabolic syndrome and the prevalence of microalbuminuria (r = 0.44, p < 0.001).⁴

Albuminuria is an early marker of CKD which also acts as a predictor of CVD and mortality. People with DMT2 with microalbuminuria, progression to massive proteinuria occurs in 20-40% of cases, while 10-50% of DMT2 patients with proteinuria will have CKD and require renal replacement therapy.²

The aim of this study was to determine the association between metabolic syndrome and albuminuria in people with DMT2 especially in Surabaya. It could give a little description of the prevalence and characteristics of metabolic syndrome in the population of type 2 diabetes, especially in Surabaya. In addition, to predict the possibility of diabetes patients with metabolic syndrome that have cardiovascular complications. Thus, the therapy can reduce the possibility of cardiovascular complications as early as possible.

Methods

This was an observational analytic study with cross sectional design. Population of this study was DMT2 patients over the age of 30 years old and less than 80 years old who undergo treatment at a private outpatient clinic Metabolic and Diabetes Endocrinologist Internist Consultant in Surabaya. The sample was 40 people, taken by using non random purposive sampling technique.

Independent variable was components of metabolic syndrom (waist size, triglyceride level, HDL level, systolic pressure, diastolic pressure, and fasting blood glucose level). Dependent variable was albuminuria, divided into normo albuminuria, mikro albuminuria, and makro albuminuria. The data was collected using medical record and was analyzed using Spearman Correlation and Mann-Whitney.

Results

Demographic and Clinical Chracteristic

The number of subjects included this study was 131 DMT2 patients, consisting of 71 men (54.2%) and 62 women (45.8%). The overall clinical characteristics of the subjects were summarized in Table 1. Median of age was 59 (minimum 31 - maximum 79) years old, median of body mass index (BMI) was 29.12 (minimum 22.48 - maximum 45.91) kg/m2, median of systolic blood pressure was 130 (minimum 100 - maximum 170) mmHg, diastolic blood pressure 80 (minimum 60 maximum 110) mmHg, median of waist circumference was 95 (minimum 80 - maximum 121) cm, median of fasting blood glucose was 160 (minimum 65 - maximum 427) mg/dL, median of 2 hours post prandial glucose was 237 (minimum 104 - maximum 749) mg/dL, median HbA1C 8 (minimum 5 - maximum 18)%, median cholesterol 198 (minimum 104 - maximum 318) mg/dL, median LDL 119 (minimum 48 - maximum 254) mg/ dL, median Triglyceride 153 (minimum 58 - maximum 484) mg/dL, median HDL 43 (minimum 13 - maximum 74) mg/dL, median Hb 14 (minimum 10 - maximum 16.2) g/dL, median BUN 15 (minimum 5 - maximum 52) mg/dL, median serum creatinine 1 (Minimum 0.4 -Maximum 1.3) mg/dL, and median Albuminuria (ACR) 14 (min imum 1 - maximum 1364) μ g/mg creat.

Table 1. Demographic and clinical characteristic

Variable	Unit	Median	Minimum	Maximum
Age	year	59	31	79
Duration of DM	year	13	1	37
BMI	kg/m2	29.12	22.48	45.91
Systolic BP	mmHg	130	100	170

Diastolic BP	mmHg	80	60	110
Waist size	cm	95	80	121
Fasting Blood Glucose Level	mg/dL	160	65	427
Post Prandi- al Glucose Level	mg/dL	237	104	749
HbA1C	%	8	5	18
Total Cholesterol	mg/dL	198	104	318
LDL	mg/dL	119	48	254
TG	mg/dL	153	58	484
HDL	mg/dL	43	13	74
Hb	g/dL	14	10	16.2
BUN	mg/dL	15	5	52
Serum Cre- atinin	mg/dL	1	0.4	1.3
Albumin- uria (ACR)	µg/mg kreat	14	1	1364

Profile of Metabolic Syndrome of the subjects

Profile of metabolic syndrome was classified by the components of the metabolic syndrome based on IDF criteria in 2006. From 131 subjects, the proportion of patients with 3 components of metabolic syndrome were 67 (50.4%) people, patients with 4 components of metabolic syndrome were 47 (35.3%) people, and patients with 5 components of metabolic syndrome were 19 (14, 3%) people, as seen in Figure 1.

Characteristics of DMT2 subjects based on classification of metabolic syndrome components are shown in Table 2. Due to the abnormal distribution of the data, non-parametric test calculation were used, and Kruskal Wallis test was chosen to compare more than 2 variables. This study found significant differences in systolic blood pressure with metabolic syndrome component group p=0.000, diastole blood pressure p=0.000, hypertriglyceridemia p=0.000, low HDL p=0.000, and albuminuria p=0.002. Meanwhile, for age, duration of DM, body mass index, waist circumference, fasting blood glucose, post prandial glucose, HbA1C, total cholesterol, LDL, Hb, BUN, and serum creatinine, there were no significant differences.

Due to the significant differences in systolic BP, diastolic BP, hypertriglyceride and low HDL with metabolic syndrome, further tests were performed with Mann-Whitney test to find out where the difference lies, whether in the 3 components group with 4 components, 3 components group with 5 components, or 4 components group with 5 components group of metabolic syndrome. Based on the Mann-Whitney test, there was a significant difference between 3 components group and 4 components of metabolic syndrome, p=0.007 in systolic BP, p=0.000 in diastolic BP, p=0.000 for hypertriglycerides, and p=0.001 for low HDL. Between

3 components group and 5 components, a significant difference was obtained with p=0.000 in systolic BP, p=0.000 in diastolic BP, p=0.000 for hypertriglyceride, and p = 0.000 for low HDL. Between groups of 4 components and 5 components of metabolic syndrome, a significant difference was obtained in systolic BP (p=0.001), whereas in diastolic BP, hypertriglyceride, and low HDL there were no significant differences.

Profile of albuminuria in patients with DM type 2 and metabolic syndrome

Albuminuria was classified based on Perkeni Consensus in 2006, consisting of normoalbuminuria, microalbuminuria, and macroalbuminuria. Albuminuria examination used ACR method. Subjects with normoalbuminuria were 87 (65.4%) people, microalbuminuria were 36 (27.1%) people, and macroalbuminuria were 10 (7.5%) people.

Characteristics of DMT2 with metabolic syndrome based on albuminuria classification (normoalbuminuria, microalbuminuria, macroalbuminuria) can be seen in Table 3. A significant difference was found in systolic blood pressure with albuminuria (p=0.024). Whereas in age, duration of DM, body mass index (BMI), diastolic blood pressure, waist circumference, fasting blood glucose (FBG), post prandial level, HbA1C, total cholesterol, LDL, triglycerides, HDL, Hb, BUN, and serum creatinine no meaningful difference obtained.

Due to the significant differences found in systolic BP, then further tests with the Mann-Whitney test was carried out. Based on the Mann-Whitney test, there was a significant difference between the normoalbuminuria and microalbuminuria groups (p=0.007). However, between normoalbuminuria and macroalbuminuria groups, there was no significant difference (p=0.362). Likewise, for microalbuminuria and macroalbuminuria groups, no significant difference was found (p=0.435).

Association between metabolic syndrome and albuminuria

The correlation between components of metabolic syndrome and albuminuria can be seen in Table 4. Spearman's test was used to measure the correlation between two unpaired variables. A significant association was found between systolic blood pressure and albuminuria, p=0.000 and r=0.325. Fasting blood glucose with albuminuria, p = 0.01 and r=0.223. On the other hand, no significant association was found between diastolic blood pressure and albuminuria, p=0.153, but a positive r value (r=0.125) means that although there was no statistically significant association, if diastolic blood pressure is higher, albuminuria will increase too. Likewise, between waist circumference with albuminuria, p=0.311 but with a positive r value (r=0.089). The same goes with hypertriglyceridemia with albuminuria, p=0.727 and r=0.031. Even so, association of low HDL and albuminuria found p=0.771 and r value =-0.025 mean that the lower of HDL will increase albuminuria.

 Table 4. Correlation between components of metabolic syndrome and albuminuria

Components of metabolic syndrome	r	р
Waist circumference	0.089	0.311
Systolic BP	0.325**	0.000*
Diastolic BP	0.125	0.153
HDL	-0.025	0.771
Triglyceridemia	0.031	0.727
Fasting blood glucose level	0.223**	0.010*

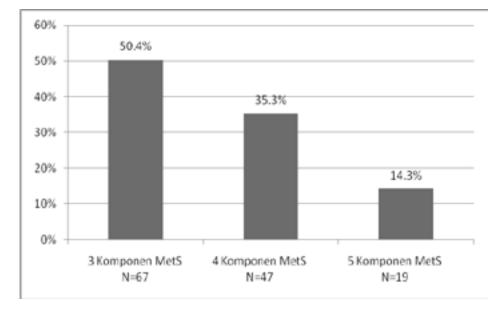


Figure 1. Proportion of metabolic syndrome based on the number of components

Variable	Unit	3 Component MetS n=19	4 Component MetS n=19	5 Component MetS n=19	Р
Age	year	58 (31-79)	64 (36-79)	59 (40-72)	0.058
Duration of DM	year	12 (2–37)	12 (1–34)	12 (3-22)	0.965
BMI	kg/m2	29.17 (24.09-40.61)	28.72 (22.48-38.02)	29.75 (24.98-45.91)	0.570
Systolic BP	mmHg	120 (100-170)	130 (110-170)	140 (130-170)	0.000*
Diastolic BP	mmHg	80 (60-95)	80 (70-110)	85 (78-110)	0.000*
Waist size	cm	94 (80-121)	96 (81-120)	95 (80-118)	0.898
Fasting Blood Glucose Level	mg/dL	152 (65-409)	177 (85-418)	188 (101-427)	0.227
Post Prandial Glucose Level	mg/dL	227 (104-749)	248 (104-555)	253 (111-641)	0.919
HbA1C	%	8 (5-18)	8 (5-113)	9 (7-12.2)	0.454
Total Cholesterol	mg/dL	191 (104-285)	198 (127-318)	221 (137-282)	0.183
LDL	mg/dL	117 (48-254)	118 (58-248)	139 (51-201)	0.273
TG	mg/dL	123 (63-356)	183 (58-484)	203 (153-388)	0.000*
HDL	mg/dL	46 (32-74)	41 (13-61)	39 (23-49)	0.000*
Hb	g/dL	14 (10-16.2)	14 (10.2-16.2)	13 (12-16)	0.598
BUN	mg/dL	15 (7.1-52)	15 (5-28)	12 (8-24)	0.859
Creatinin serum	mg/dL	1 (0.4 – 1.24)	1 (0.60-1.3)	1 (0.4-1.1)	0.404
Albuminuria	µg/mg kreat	14 (1-983)	7 (1-183)	122 (1-1364)	0.002*

Table 2. Characteristics of DMT2 with metabolic syndrome based on the classification of metabolic syndrome components

Table 3. Characteristics of DMT2 with metabolic syndrome based on albuminuria classification

Variable	Unit	Normo albumin-	Mikro albuminuria	Makro albumin-	Р
		uria n=87	n=36	uria n=10	
Age	year	59 (33-79)	63 (31-75)	57 (40 - 69)	0.330
Duration of DM	year	12 (1–37)	12 (1–32)	10.5 (3-22)	0.884
BMI	kg/m2	29 (22.48-38.02)	29.53 (24.98-45.91)	28.32 (25.20-34.46)	0.300
Systolic BP	mmHg	125 (100-165)	132.5 (100-170)	130 (110-160)	0.024*
Diastolic BP	mmHg	80 (60-100)	80 (60-110)	80 (70-110)	0.296
Waist size	cm	94 (80-121)	95 (80-114)	101 (90-118)	0.321
Fasting Blood Glucose Level	mg/dL	161 (64-418)	159.5 (78-427)	159.5 (118-409)	0.807
Post Prandial Glucose Level	mg/dL	248 (104-555)	230.5 (111-749)	237 (129-569)	0.906
HbA1C	%	8 (5.7 -14.2)	8.75 (5-18)	8.1 (6-12)	0.254
Total Cholesterol	mg/dL	197 (107-318)	208 (104-285)	181.5 (160-267)	0.441
LDL	mg/dL	115 (58-248)	128.5 (51-254)	113.5 (48-150)	0.076
TG	mg/dL	153 (58-484)	158.5 (79-361)	156.5 (102-256)	0,970
HDL	mg/dL	42 (13-74)	44 (23-66)	44.5 (36-56)	0.881
Uric Acid	mg/dL	5.7 (2.4-9.4)	5.5 (3.3-9.7)	5.65 (3.1-7.5)	0.997
SGOT	mg/dL	25 (8-59)	29.5 (9-62)	21 (13.1-51)	0.575
SGPT	mg/dL	28 (10-69)	29.5 (12-66)	25.5 (14-45)	0.831
Hb	g/dL	14 (10-16.2)	13.9 (10.2-16.2)	13.8 (12-14.5)	0.764
BUN	mg/dL	15 (5-36)	12.8 (6.3-52)	15.5 (7.1-23)	0.778
Serum Creatinin	mg/dL	1 (0.4 – 1.3)	1 (0.40-1.21)	1 (0.7-1)	0.390

Discussion

Metabolic syndrome in DM type 2

This study used IDF criteria in 2006 to determine metabolic syndrome. The results found that patients with 3 components of metabolic syndrome were 50.4%, patients with 4 components of metabolic syndrome were 35.3%, and patients with 5 components of metabolic syndrome were 14.3%. Meanwhile, the study by Ford, et al. found that the prevalence of individuals with one component of metabolic syndrome was 71.2%, two components 43.9%, three components 23.7%, four components 10.4%, and five components 2.7%.⁵ Lea reported the component profile of the metabolic syndrome was 7.1% with one component, 92.9% with two components, 63.1% with three components, 33.3% with four components, and 13.1% with 5 components.⁶

The prevalence of metabolic syndrome is influenced by factors that can or cannot be modified. Many of these factors are interrelated, including age, gender, ethnicity/ race, obesity and fat distribution, diet and physical activity patterns, birth weight, genetics, endocrine factors, menopause or hormone replacement therapy, inflammation, alcohol, and comorbid factors (such as diabetes, hypertension, coronary artery disease). Nevertheless, the prevalence of metabolic syndrome can be increased due to the increasing prevalence of obesity and central obesity.⁷

Jorgenson examined the prevalence of metabolic syndrome in a population over 35 years old, found two different figures using different criteria, using WHO criteria the prevalence was 20.7%, while using NCEP-ATP III criteria the prevalence was 17.9%.⁸ Pranoto, et al. reported that the prevalence of metabolic syndrome based on NCEP-ATP III criteria was 34%.⁹ This is similar to the study by Adam, et al. that prevalence of metabolic syndrome was 33.4%.¹⁰ The diagnosis of metabolic syndrome is based on the criteria of NCEP ATP III which are modified for Asian populations, if there are 3 out of the 5 criteria listed below: 1.Waist size for male > 90 cm and female > 80 cm,

2. Increases of triglyceride level > 150 mg/dL,

3.Decreases of HDL cholesterol level on male < 40 mg/dLand female < 50 mg/dL,

4. Increases of blood pressure > 130/85 mmHg,

5.Increases of fasting blood glucose level > 110mg/dL or having diabetes.²

In contrast to the criteria for metabolic syndrome according to IDF in 2006, which stated a person has metabolic syndrome if he/she has central obesity (characterized by waist circumference > 90 cm in male and > 80 cm for female) plus 2 or more than 4 of the following components:

1.Triglyceride level > 150 mg/dL or being fat disorder treatment,

2.HDL level < 40 mg/dL on male and < 50 mg/dL on female or being fat disorder treatment,

3.Systolic blood pressure > 130 mmHg or diastolic > 85 mmHg or being hypertension treatment,

4.Fasting blood glucose level > 100 mg/dL or being DM type 2 treatment.¹

The proportion for each component of metabolic syndrome in this study found 52.6% subjects with

hypertension, 56.4% subjects with hypertriglyceridemia, 54.9% subjects with low HDL cholesterol, 100% central obesity, and 100% diabetes mellitus. This study shows that all subjects have central obesity, this is because in determining the metabolic syndrome this research used IDF criteria in 2006 where a person is said to have metabolic syndrome if he/she has central obesity marked by waist circumference > 90 cm in men and > 80 cm for women, plus 2 or more than 4 other components of metabolic syndrome. All subjects in this study also had diabetes mellitus because the subject population was taken from people with DMT2.

These results are similar with other researches. Research conducted by Pranoto, et al. reported the proportion of central obesity of 85.29%, hypertriglyceridemia 85.29%, low HDL cholesterol 52.94%, increased systolic blood pressure 82.35%, increased diastolic blood pressure 55.88%, and increased fasting blood glucose 41.17% (9). Meanwhile, research conducted by Michel in 2006 on 476 metabolic syndrome populations found central obesity (14.3%), hypertriglyceridemia (17.0%), increased blood pressure or those using ant1 hypertension (80.7%), increased blood glucose levels fasting or those using anti-diabetes therapy (37.2%).¹¹

Albuminuria in DM type 2

The results from 133 DMT2 patients, 65.4% of patients have normoalbuminuria, 27.1% microalbuminuria, and 7.5% macroalbuminuria. It is in line with research by Tseng on 549 DMT2 patients, which found 45.7% DMT2 patients with normoalbuminuria, 44.1% patients with microalbuminuria, and 10.2% patients with macroalbuminuria, found 63.4% patients with normoalbuminuria, 27.5% patients with microalbuminuria, and 9% patients with macroalbuminuria.¹³

Microalbuminuria can be an etiology or complication of vascular disease. STENO hypothesis said that leakage of albumin into the urine is a reflection of extensive vascular damage. This hypothesis links endothelial dysfunction with vascular albumin leakage. In some ways, the kidneys are the window to the body's vascular system. Renal leakage can reflect the permeability of the overall vascular system.¹⁴ A significant difference was found in systolic blood pressure with albuminuria p = 0.024. Whereas in age, duration of DM, body mass index (BMI), diastolic blood pressure, waist circumference, fasting blood glucose (FBG), post prandial glucose level, HbA1C, total cholesterol, LDL, triglycerides, HDL, Hb, BUN, and serum creatinine no meaningful difference obtained. Based on the Mann-Whitney test, there was a significant difference in systolic blood pressure between the normoalbuminuria and microalbuminuria groups, p = 0.007. However, between normoalbuminuria and macroalbuminuria groups, there was no significant difference. Likewise, for microalbuminuria and macroalbuminuria groups, no significant difference was found.

Published data from cross sectional studies show the prevalence of microalbuminuria in hypertensive populations ranging from 4.7% to 40%, depending on the severity of hypertension and comorbidity. Research by de Melo¹⁵ confirmed the Nogueira study, which showed that there was an association between blood pressure changes and the incidence of diabetes mellitus with an increase of albuminuria in individuals with hypertension. This suggests that efforts to achieve adequate blood pressure and glycemic control must be sought, with the aim of reducing albuminuria excretion and consequently preventing evolution towards target organ damage and decreasing morbidity and mortality.¹⁵

Correlation between metabolic syndrome and albuminuria

This study found a significant association between systolic blood pressure and albuminuria, p = 0.000 and r = 0.325. Fasting blood glucose with albuminuria, p = 0.01 and r = 0.223. This is consistent with the results shown by research conducted by Hao, et al.⁴ as well as by Palaniappan, et al.¹⁶ Whereas in diastolic blood pressure, waist circumference, low HDL, and hypertriglyceridemia, there was no significant association.

Study by Hao, et al. showed a significant relationship between metabolic syndrome and albuminuria. This study also stated that blood pressure and obesity individually have an association with ural microalbumin.⁴ In the study of Palaniappan, et al., a strong positive relationship was found between the components of metabolic syndrome and microalbuminuria in both male and female populations. From this research it shows that high glucose levels and blood pressure are the most influential components on the occurrence of microalbuminuria.¹⁶ Study by Tseng showed that TG levels increased with increasing albuminuria. TG levels differed significantly between the three groups of albuminuria and correlated with albuminuria (ACR) for the entire albuminuria range.¹²

Numerous studies have shown that lipid abnormalities can initiate glomerular injury and increase the development of existing glomerular disease. The final pathway for most of these diseases is generally the development of glomerulosclerosis. The mechanism that associate hypercholesterolemia with the development of glomerular disease is thought to be the same as the influence of high cholesterol levels which cause atherosclerosis.¹⁷

Glomerular cells have LDL receptors, mesangial, and glomerular epithelial cells that can internalize atherogenic lipoproteins through receptor or non-receptor intermediate mechanisms. Infiltration of atherogenic lipoproteins into glomerular endothelium and mesangial cells can initiate a cascade of events including expression of adhesion molecules, production of monoattractant monocytes, and reactive release of oxygen species, resulting in initial glomerular injury.¹²

In the study of Zoppini with a large 5-year cohort study involving 1987 outpatient DMT2 patients, it showed that high plasma HDL levels were strongly associated with a reduction in the incidence of CKD (GFR ≤ 60 mL / min / 1.73 m2). Similar results in a study by Ravid showed low plasma HDL levels predicted the development of microalbuminuria in 574 people with DMT2 during a 7-8 year follow-up.¹⁸

The biological mechanisms underlying HDL in protecting the development and progression of CKD in people with DMT2 are poorly understood. Actually, HDL is involved in reverse transportation (reversal) of cholesterol, but HDL can also use several other effects, including antioxidants, anti-inflammatory, anti-thrombotic effects and protective effects of blood vessels. All of these beneficial vascular effects can also play a role at the level of kidney microcirculation, so it might explain the lower risk of CKD in the presence of higher HDL levels.¹⁸

Finally, pharmacological interventions that are known to be effective in preventing or delaying the development of cardiovascular disease in people with DMT2, including close monitoring of blood pressure, LDL levels and glucose, can reduce the development and progression of kidney disease. The potential clinical implications of this finding for patient care is that in people with DMT2 with low plasma HDL levels, physicians must be more aware of the greater possible risk for developing CKD, and that pharmacological treatment aimed specifically at increasing plasma HDL levels may be a promising approach to reducing risk of development and progression of kidney disease.

Conclusion

There is a significant association between systolic blood pressure and fasting blood glucose level with albuminuria. Whereas in diastolic blood pressure, waist circumference, low HDL, and hypertriglycerida, there was no significant association. In the future, it is necessary to control the components of the metabolic syndrome, so that the risk for cardiovascular disease complications marked by albuminuria can be reduced. The high proportion of microalbuminuria in DMT2 patients has led to the need for increased attention from health care providers to conduct microalbuminuria screening tests for each DMT2 patients.

Conflict of Interest

The author stated there is no conflict of interest

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