ORIGINAL ARTICLE

Title

Correlation between Urinary Cyclophilin A and Urinary Albumin Levels on Diabetic Kidney Disease

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Editor:
Syakib Bakri

Received 19 March 2019, revised 11 July 2019, accepted 17 July 2019, published 1 August 2019

Abstract

Background: Diabetic kidney disease (DKD) is a complication of diabetes mellitus characterized by albuminuria persisting within 3 to 6 months, the earliest clinical evidence is microalbuminuria (30-299 mg/24hours or 20-199 ug/i). Cyclophilin A (Cyp A) is an 18 kDa 165-amino acid long cytosolic protein also known as peptidylprolyl isomerase A. In DKD, hyperglycemia will cause Cyp A secretion by human kidney-2 (HK-2) cells from PTEC and mesangial-13 cells (MES-13) and causes kidney damage.

Objectives: To elucidate the correlation between urinary cyclophilin A and urinary albumin levels in patients with DKD.

Material and Methods: This study was an analytic observational, cross-sectional study conducted at the clinic and inpatient internal medicine installation at dr. M. Djamil General Hospital, Padang for 6 months. Samples were selected by consecutive sampling, as many as 60 people with postprandial blood glucose > 180 mg/dl and urinary albumin > 30 mg/24 hours and met the inclusion and exclusion criteria. The samples were examined for urinary Cyp A and albumin levels.

Results: The mean level of urinary Cyp A in patients with DKD is 4.96 (2.03) ng/ml. Median urinary albumin levels in DKD patients is 287.89 (30.79-394.57) mg/24 hours. Correlation analysis between urinary Cyp A and albumin levels showed a significant ($p < 0.05$) with a positive and strong correlation ($r = 0.776$) in DKD patients.

Conclusion: There was an increase of urinary Cyp A and urinary albumin levels, with a positive and strong correlation between them in DKD patients.

Keywords: Urinary Cyclophilin A, urinary albumin, diabetic kidney disease

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Introduction

Diabetic kidney disease (DKD) is a health problem throughout the world including Indonesia. DKD is

a complication of diabetes mellitus (DM) which is characterized by albuminuria persisting through at least two examinations within 3 to 6 months, where the earliest clinical evidence of DKD is microalbuminuria (30 - 299 mg/24hours or 20 - 199 ug/i).[1]

According to the World Health Organization (WHO), the prevalence of DM will increase throughout the world in this third millennium. Data from WHO in 54 countries showed the prevalence of DKD has an average of 55%.[2] Indonesian Renal Registry (2015) obtained the data that DM was the second largest etiology (25%) after hypertension, where the incidence of DKD in West Sumatra had a percentage of 28.6% of all CKD events registered in the dialysis unit.[3]

The pathogenesis of DKD is the activation of metabolic pathways, increased production of proinflammatory cytokines, and hemodynamic pathways. The metabolic pathway due to chronic hyperglycemia will cause nonenzymatic glycosylation which causes an increase and accumulation of advanced glycation end products (AGEs). In addition, it also activates the signaling pathways such as diacylglycerol-protein kinase C (DAG-PKC) pathway, polyol pathway, and hexosamine. The effect of metabolic pathway action and the production of inflammatory mediators will cause glomerular and tubular disturbances which will end in decreasing the glomerular filtration rate (GFR) and proteinuria.[4]

In addition to activation of the metabolic pathway and the activation of profibrotic cytokines and hemodynamic pathways, there is another pathway that causes DKD, cyclophilin A (Cyp A). Cyclophilin A has a role in the occurrence of tubulointerstitial fibrosis. Cyclophilin A is an 18 kDa protein 165-amino acid long cytosolic protein, also known as peptidylprolyl isomerase A, is a distributed protein in almost every part of the body. Cyclophilin A can regulate various biological processes such as intracellular signaling, transcription, inflammation, and apoptosis.[5] Cyclophilin A is secreted rapidly and directly by mesangial cells and proximal tubular epithelial cell (PTEC) after hyperglycemia.[5]

Hyperglycemia will induce the secretion of Cyp A, the highest secreted by human kidney-2 (HK-2) cells from PTEC because Cyp A expression is at a relatively high level in PTEC compared to other kidney cells. Apart from tubular changes, the histological changes of mesangial cells in DKD will cause Cyp A secretion from mesangial-13 (MES-13) cells in mesangial.[6]

Tsai et al (2015) in his research on human studies found urinary Cyp A level correlated with the development of kidney function based on albuminuria levels. The study showed that when the concentration of urinary Cyp A was more than 0.7250-2 ng/ml, DKD stage 2 could be diagnosed with a sensitivity of 90.0% and specificity of 72.7%.[5] In cellular studies with given MES-13 and HK-2 cells with high glucose and H_2O_2 treatment increased expression of Cyp A. The Tsai study concluded that urinary Cyp A is a good biomarker for early DKD detection in humans, can be released from mesangial or tubular kidney cells, and has a role in the pathophysiology of DKD.[5]

The earliest clinical evidence of DKD is microalbuminuria (30-299 mg/24hours or 20-199 ug/i) which is called incipient nephropathy. Without specific interventions, albumin excretion will increase by 10-20% per year and will eventually become overt nephropathy (> 300 mg/24hours or > 200 ug/i) within 10-15 years. If overt nephropathy has occurred, without specific intervention, GFR will gradually decrease over several years with different variations between individuals (2-20 ml/year) which will end in terminal kidney failure.[7]

Based on the background described above, a study was conducted on the correlation between urinary cyclophilin A and urinary albumin levels in patients with DKD to determine the role of cyclophilin A in the process of kidney damage in DKD.

Materials and Methods

This was an analytic observational study with cross-sectional approach conducted in outpatients and inpatient internal medicine installation of Dr. M. Djamil Hospital Padang for 6 months. The population of the study was patients with DKD. The inclusion criteria were patients with DM with postprandial blood glucose > 180 mg/dl dan urinary albumin >30 mg/24 hours. The exclusion criteria were fever, doing heavy exercises one day prior to urinary sample collection, rheumatoid arthritis, systemic lupus erythematosus, urinary tract infections, hepatic cirrhosis, cardiovascular disease, and currently on immunosuppressant therapy. The sample size was calculated using the coefficient correlation formula and the sample size needed was 60 patients.

Diabetic kidney disease was defined as patients with diabetes and post-prandial blood glucose exceeding 180 mg/dl and albuminuria > 30 mg/24 hours. Albuminuria was measured using enzyme-linked immunoabsorbent assay (ELISA) and categorized into normal (<30 mg/24 hr), microalbuminuria (30-300 mg/24hr), and macro-albuminuria (>300mg/24 hr). Urinary cyclophilin -A was also measured using ELISA, categorized into normal (<0.725 ng/dl) and elevated (≥0.725 ng/dl). Two milliliters of 24-hour urine collected was used for laboratory examination.

Results and Discussion

Results

Table.1 presents the characteristic of 60 DKD patients. Characteristics include age, blood pressure, body mass index, dyslipidemia, and fasting blood sugar levels. In this study, the average age was 55.83 (8.25) years. Average body mass index is 23.30 (2.88) kg.m^2. In this study, 60% of subjects were classified as overweight and obese. The number of patients with hypertension in this study was 63.3%. On examination of the lipid profile, most patients were classified as dyslipidemia, which amounted to 56.7%. The mean postprandial blood glucose is 234.86 (50.10).

Table.1 Characteristic of sample

Characteristic	N (%)	Mean (SD)
Age (year)		55.83 (8.25)
<60	40 (66.7)	
≥60	20 (33.3)	
BMI (kg/m^2)		23.30 (2.88)
Normal	24 (40)	
Overweight and obese	36 (60)	
Hypertension		
Yes	38 (63.3)	
No	22 (36.7)	
Triglyserides (mg/dl)		159.53 (55,35)
<150	26 (43.3)	
≥150	34 (56.7)	
Postprandial blood glucose (mg/dl)		234.86 (50,10)
<230	34 (56.7)	
≥230	26 (43.3)	

The mean level of urinary cyclophilin A in patients with DKD is 4.96 (2.03) ng/ml with the lowest levels was 1.07 ng/ml and the highest was 8.36 ng/ml. The results of the Shapiro Wilk normality test showed that urinary cyclophilin A level in this study were normally distributed (p> 0.05).

Median urinary albumin level in patients with DKD was 287.89 (30.79-394.57) mg/24 hour with the lowest urinary albumin level was 3.79 mg/24 hours and the highest is 394.57 mg/24 hour. The results of the Shapiro Wilk normality test showed that urinary albumin level in this study was not normally distributed (p <0.05) as shown in Figure.1.

Correlation analysis between urinary cyclophilin A and albumin levels used Spearman correlation test. The results showed a significant correlation between urinary cyclophilin A and albumin in patients with DKD (p <0.05) with a positive and strong correlation (r = 0.776).

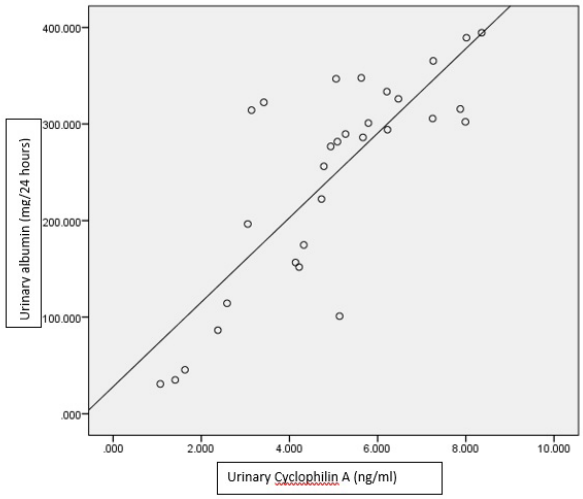

Figure.1 Correlation between urinary cyclophilin A and urinary albumin levels in patients with DKD

Discussion

The mean age obtained from this study was 55.83 (8.25) years. The mean age of DKD patients from Behradmanesh et al (2013) was 57 (8.3) years.[8] Tsai et al study (2015) found that the average age of patients with DKD was 57.3 (11.6) years.[6] While the study from Amer et al (2018) in 60 DKD patients, the mean age was 53.03 (8.09).[9]

Chowta et al (2009) conducted a study of 100 people with type 2 diabetes found a significant correlation between age and incidence of albuminuria. From existing epidemiological studies, it has been proven that the incidence of albuminuria increases with age.[10]

The average body mass index (BMI) in this study was 23.30 (2.88) kg/m^2. Most subjects are classified as overweight and obese (60%). Noha et al (2012) also showed a high average BMI of 29.9 kg/m^2.[11] Study of Amer et al (2018) found a mean BMI in DKD patients was 26.34 (3.96) kg/m^2.[9] This is in accordance with the study of Masahiko T (2008) who found that body mass index significantly correlated with the incidence of albuminuria.[12]

Yassamine et al study (2013) found that BMI does not directly cause a decrease in GFR and risk factors that cause a decrease in kidney function in DKD patients differ from each other. A decrease in GFR and an increase in urinary albumin is important as a marker of DKD severity, so this will lead us to include groups with high BMI as a consideration in the management of DKD.[13]

Patients with hypertension were more than patients without hypertension (63.3%). Chul-Woo et al study (2011) found that the incidence of hypertension was 76.05% in DKD patients.[14] Hsu et al study (2012) also found a greater number of DKD patients with hypertension, about 56.1% .[15] Tsai et al study (2015) found patients with hypertension in DKD 6 was 5.65% .[6] While studies from Amer et al (2018) obtained 68.37% of DKD patients with hypertension. [9]

Based on this study and other studies, it can be concluded that most DKD patients have hypertension. The mechanism of the occurrence of hypertension in DKD is complex and not yet fully understood. Its main causes include volume expansion due to increased renal sodium reabsorption and peripheral vasoconstriction due to dysregulation of the factors that regulate peripheral vascular resistance, RAAS activation, an increase of ET-1 regulation, ROS regulation and decrease of NO regulation. Many pathogenic factors that affect local non-hemodynamic effects can accelerate the occurrence of DKD. [16]

The proportion of patients with dyslipidemia was also higher (56.7%). The mean triglyceride levels in this study were 159.53 (55.35) mg/dl. Syrjanen et al (2000), Masahiko T et al (2008) and Nakhjavani et al (2008) found a significant relationship between dyslipidemia and albuminuria. [12,17,18] Based on consensus from PERKENI (2015), dyslipidemia is often seen in DM patients are elevated triglycerides (> 150 mg/dl), decreased HDL (<40 mg/dl) and normal or slightly elevated LDL cholesterol level.[19] Triglycerides have a lipotoxicity effect which will cause long-chain fatty acids to be delivered to the kidneys via serum albumin

and stored in the kidney cells and tubules. This will cause tubulointerstitial inflammation and fibrosis in mild cases and kidney failure and death in severe cases. Triglycerides will be transported from VLDL to HDL which is rich in TG particles, which will then be hydrolyzed by the liver lipase enzyme and will be eliminated from the plasma.[20]

Mean of postprandial blood glucose level was 234.86 (50.10) mg/dl. Based on PERKENI (2015) and ADA (2015) fasting and postprandial blood glucose levels is one of the criteria for controlling diabetes mellitus.[19] Whoerle et al (2007) found, when the fasting blood glucose level was on target, only 64% of patients reached HbA1C levels ≤ 7%, and when fasting and postprandial blood glucose was in target levels, 94% of patients achieved HbA1C level ≤ 7%. This was also obtained from the study of Monnier et al (2003). The postprandial blood glucose was more pronounced in the long-term micro and macrovascular complications abnormalities while fasting blood glucose shows the initial progression of DM.[21] Tsai et al study (2015) showed the mean fasting blood sugar in DKD patients was 142.9 (50.5) mg/dl.[6] While studies from Amer et al (2018) showed the mean of fasting blood sugar levels in DKD patients were 176.33 (88.90) mg/dl. Based on the results of these studies it was concluded that the condition of hyperglycemia can cause kidney damage in patients with diabetes mellitus.[9]

The condition of hyperglycemia will cause cyclophilin A to be secreted from mesangial cells and excessive tubular cells. This Cyclophilin A will later bind to CD147 as its receptor and cause activating p38, which will later cause Epithelial Mesenchymal Transition (EMT) reactions via p38 MAPK signaling pathway which will cause glomerulosclerosis and tubulointerstitial fibrosis.

The mean level of urinary cyclophilin A was 4.96 (2.03) ng/ml. Urinary cyclophilin A level of 1.07 ng/ml can be detected at albumin levels 30.79 mg/24 hours or in conditions of microalbuminuria. Tsai et al study (2015) obtained a mean level of urinary cyclophilin A was 3.16 (5.36) ng/ml where DKD stage 2 can be detected in levels of urinary cyclophilin A 0.7250 ng/ml. [6] Amer et al (2018) found that the mean level of urinary cyclophilin A in microalbuminuria patients was 4.79 (1.25) ng/ml and in patients with macroalbuminuria was 7.23 (0.76) ng/ml. The level of urinary cyclophilin A was 1.69 (0.87) in DKD stage 2 patients. The conclusion is that urinary cyclophilin A level began to increase significantly in DKD stage 2 and higher levels in higher DKD stages.[9]

Hyperglycemia is an initial process that causes structural and functional changes of the kidneys, such as glomerular hyperfiltration and microalbuminuria, which lead to overt proteinuria and eventually end-stage kidney disease. In this study, it was found that the median of subjects' urinary albumin level was 287.89 (30.79-394.57) mg/24 hours. Kundu D et al found the average ACR level in DKD patients was 449 (160) ug/mg creatinine. [22] Dizin et al (2013) obtained an average ACR level was 528.9 (165.4) ug/mg creatinine. [23] Aziz KM (2015) obtained an average ACR level in patients with DKD was 78.1 (109.3) ug/mg creatinine.[23] The difference in urinary albumin level in this study might be to abnormally distributed data because the subjects in this study were taken from all stages of chronic kidney disease.

Correlation analysis of urinary cyclophilin A with albumin level was used by Spearman correlation test with confidence level $p < 0.05$. The results of the analysis showed a significant correlation between levels of urinary cyclophilin A and albuminuria with a positive and strong correlation ($p = 0.001$, $r = 0.776$). This shows that there is an increase in urinary cyclophilin A level along with increased albuminuria.

Tsai et al study (2015) found significant differences ($p < 0.001$) of urinary cyclophilin A level in all DKD groups except in DKD stage I. This study also found

an increase in urinary cyclophilin A 0.030 ng/ml each increase of albumin-creatinine-ratio (ACR) of 1 ug/mg creatinine with linear R2 0.054.36. Amer et al who also examined the correlation of urinary cyclophilin A with urinary albumin found a statistically significant ($p < 0.05$) and very strong correlation ($r = 0.93$) in DKD patients.[9]

Based on this study and other studies examining the correlation between urinary cyclophilin A and albumin levels in DKD patients, it was found that urinary Cyclophilin A had a positive correlation with albuminuria. This condition is caused by hyperglycemia-induced secretion of Cyclophilin A which can cause damage to the glomerular and tubular cell, and it is one of the proteins that cause an increase of albuminuria.

Conclusion

There was an increase of urinary Cyp A and urinary albumin levels, with a positive and strong correlation between them, in DKD patients.

Conflicts of Interest

There are no conflicts of interest regarding the publication of this paper.

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