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Urinary Epithelial Sodium Channel (EnaC) Level as A Diabetic Marker of Nephropathy in Type 2 Diabetes Mellitus with Hypertension

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Abstract

Increased prevalence of diabetes mellitus (DM) leads to the increased of various complications including diabetic nephropathy, which can lead to the end stage renal disease (ESRD). The Epithelial sodium channel (ENaC), which is located in distal convoluted tubules, plays an important role in transepithelial sodium reabsorption for electrolyte homeostasis. Diabetes mellitus can cause changes in ENaC function which will interfere with kidney blood pressure control, worsening hypertension, and kidney injury that eventually may trigger diabetic nephropathy. The aim of this study was to determine the validity of urinary ENaC for screening diabetic nephropathy in type 2 diabetes mellitus (T2DM) with uACR as the gold standard. This was a cross-sectional analytical observational study conducted in Dr. Hasan Sadikin General Hospital Bandung, Indonesia, from December 2020 to July 2021. The subjects were 87 patients T2DM with hypertension with the majority of subjects (n=62) had a mean age of 56 years old and were experiencing albuminuria and hyperglycemia with DM for a duration of < 10 years. The poor glycemic control in these patients accelerated the occurrence of kidney damage. Result showed that urine ENaC level had a sensitivity and specificity of 82.3% and 48%, respectively, with 72.4% accuracy. The cut-off point of urine ENaC in this study was 0.98 ng/mL. Hence, urine ENaC level can be used as a test to screen for diabetic nephropathy with 82.3% sensitivity.

Keywords: Diabetic nephropathy, ephitelial sodium channel (ENaC), urinary albumin-creatinine ratio (uACR)

Introduction

Chronic kidney disease (CKD) is a non-communicable disease that needs attention because it has become a global public health problem with a high incidence, poor prognosis, and high costs. Diabetes mellitus (DM) is a metabolic diseases characterized by hyperglycemia that occurs due to defects in insulin secretion, insulin action or both.[1] The Centers of Disease Control Prevention (CDC) in 2019, reported that around 38% of the causes of ESRD were type 2 diabetes mellitus (T2DM) will develop into diabetic nephropathy in years. Hypertension is a comorbid disease that often occurs in DM patients and interacts synergistically in increasing kidney injury in diabetic nephropathy. Diabetic nephropathy is a microvascular complication and being main cause of end stage renal disease (ESRD).[1,2] Long term diabetes may cause impaired nephron function due to structural changes, such as focal tubular atrophy, thickening of basement membrane in glomerulus and tubular, and glomerulosclerosis.[2,3]

The consensus of the American Diabetes Association (ADA) and the Indonesian Endocrinology Association (PERKENI) in 2019, states that the gold standard for diagnosing diabetic nephropathy is an examination of urinary albumin creatinine ratio (uACR) with a urine sample. Persistent urinary albumin creatinine ratio in the range of 30–299 mg/g is an early sign of diabetic nephropathy in T2DM.[1] Detection albuminuria in T2DM have a several limitation such as 30% of diabetic nephropathy patients experience normoalbuminuria and albuminuria can be detected after significant kidney injury.[4] Epithelial sodium channel (ENaC) which is a transmembrane protein consisting of 3 subunits, αENaC, βENaC, and γENaC which are located in the distal convulated tubules, cortical collecting duct, and medullary collecting duct. Epithelial sodium channels play an important roles in transepithelial sodium reabsorption in the connecting tubules and collecting ducts of the kidney for electrolyte homeostasis and

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extracellular volume so that it control blood pressure.[4] Several studies have stated that there are various mechanisms by which DM can cause changes in ENaC function that will interfere with kidney blood pressure control, worsening hypertension, and cause kidney injury that plays a role in the development of diabetic nephropathy.[5]

The aim of this study to examine the validity of urinary ENaC in patients with risk factors for CKD such as T2DM and hypertension serves to detect a decline in kidney function early and as a selection of appropriate hypertension drug therapy to prevent diabetic nephropathy. So that in the future it is expected to become an early diagnostic tool for diabetic nephropathy.

Methods

This study was an observational analytic study with cross-sectional study design which was conducted in December 2020–July 2021. The subjects of this study were male and female patients who had been diagnosed with T2DM with hypertension according to PERKENI criteria by clinicians at the Endocrine Polyclinic, RSUP Dr. Hasan Sadikin Bandung with eGFR 60–≥90 mL/min/1.73 m^2. The exclusion criteria are patient with urinary tract infection, heavy activity, smoker; subjects with primary hypertension, primary hyperaldosteronemia, and congestive heart failure can increase ENaC activity; subjects with kidney disorders such as nephrotic syndrome can increase the activity of ENaC.[6,7] Subjects with primary hypertension, primary hyperaldosteronemia, and congestive heart failure were excluded from this study by taking anamnesis regarding onset of hypertension, especially at a young age which leads to primary hyperaldosteronemia, family history of hypertension, use of >3 kinds hypertension drugs can leading to drug-resistant hypertension in primary hyperaldosteronemia, a history of seizures and limb weakness due to hypokalemia in primary hyperaldosteronemia, also a history of congestive heart failure symptoms characterized by easy fatigue and shortness of breath during light to heavy physical activity.[7] A total 2 mL Blood was collected by phlebotomy on cubital vein for creatinine examination, while urine used was ± 20 mL mid-stream urine from random urine for ENaC dan uACR examination. Examination of urine ENaC level was carried out using Sandwich ELISA method. Urine examination material of ±10 mL for ENaC examination was then centrifuged for 20 minutes at 1,000×g, the urine supernatant was separated and put into a plastic microtube of 500 L and stored at -80°C with a stability of 2 months.[8] Examination of uACR level used Meditape II 10K strip test with a semi-quantitative method, examined immediately within 1–2 hours after the samples arrived. Serum creatinine level was measured using kinetic Jaffe method. After obtaining serum creatinine result, eGFR was calculated with CKD-EPI method. Material was examined at the Central Laboratory of the Clinical Laboratory Installation of Dr. Hasan Sadikin, Bandung. Sampling was done by consecutive sampling. Sample size formula for this diagnostic test was:

$$N = \frac{(Z_\alpha)^2 . Sen.(1 - Sen)}{d^2 . P}$$

Note:
n = Number of subjects
Zα / 2 = Z value from normal distribution Table for 95% confidence interval (Z = 1.96)
Sen = The expected research sensitivity is 90.0% or 0.90 (determined by the researcher)
d = Study precision (10%)
P = Study prevalence (50%)[9]

Based on the above formula obtained:

$$N = \frac{(Z_\alpha)^2 Sen(1-Sen)}{d^2 P} \rightarrow N = \frac{(1,96)^2 0,9\,(1-0.9)}{0,1^2\,0,5}$$

$$N = 76,06 \approx 77$$

Anticipating data loss, number of samples was added to 10% of sample size, then minimum sample size was 85.

Normality test for sample of more than 50 people used Kolmogorov Smirnov test and Shapiro Wilk's test for sample less than 50 people. Normality test was declared to be normally distributed if p >0.05. For normally distributed data using parametric analysis by unpaired t test and for data that were not normally distributed using non-parametric data analysis by Mann Whitney's test and chi square. Data analysis was performed using SPSS for windows version 24.0 program at 95% confidence interval and statistically significant if p <0.05.The ENaC urine data obtained will be analyzed with ROC curve to obtain a cut-off value for the diagnosis of diabetic nephropathy and calculate the area under the curve (AUC), then analyzed the validity value to uACR gold standard.[9] This study was approved by the Health Research Ethics Committee of

Dr. Hasan Sadikin General Hospital Bandung through the issuance of the ethical approval no. LB.02.01/X.6.5/11/2021.

Results

A total of 90 people T2DM with hypertension according to the PERKENI criteria by clinicians at Endocrine Polyclinic Dr. Hasan Sadikin Bandung was included in this study. A total of 3 people were excluded from the study due to insufficient urine sample for examination. Number of subjects in this study was 87 people who met the inclusion and exclusion criteria and were willing took part in the study by signing consent form after being given an explanation (informed consent).

Characteristics of subjects based on sex, age, duration of diabetes, fasting blood glucose, blood pressure, eGFR calculation result, and ENaC level are shown in Table 1. In this Table, study subjects are classified according to normoalbuminuria (uACR level <30 mg/gCr) and albuminuria (uACR level >30 mg/gCr).

From Table 1, the mean age in the albuminuria group was ±56 years and the longest duration of DM was ≤10 years. On the characteristics of fasting blood glucose level, a subject on albuminuria group (73.6%) and normoalbuminuria (26.4%) experienced an increase in fasting blood glucose level >130 mg/dL and included into the criteria not achieving therapeutic target. Mean different test urine ENaC levels between the groups normoalbuminuria and albuminuria was found that the average level of ENaC urine in albuminuria group of 1.90 ng/mL, whereas in the group normoalbuminuria 0.99 ng/mL.

Table 2 shows sensitivity and specificity value of urine ENaC level of various cut-off value. Cut-off value used in this study was 0.98 with sensitivity and specificity, 82.3% and 48%, respectively.

Figure 1 shows the ROC curve of urine ENaC level. From cut-off value of 0.98, area under the curve (AUC) was found to be 81%.

In Table 3, sensitivity and specificity value obtained were 82.3% and 48%, respectively. At the cut-off of 0.98 ng/mL, a high sensitivity value of 82.3% showed that there was an 82.3% possibility that the urine ENaC level gave a result >0.98 ng/mL in T2DM subjects with albuminuria. The specificity value of 48% indicates that there is a 48% probability that the urine ENaC level will give a result of 0.98 ng/mL in T2DM subjects with normoalbuminuria. A positive predictive value of 79.7% indicates that there is a 79.7% possibility of T2DM patients experiencing albuminuria if the urine ENaC level is >0.98 ng/mL. The negative predictive value is 52.2%, meaning that there is a 52.2% possibility of T2DM patients experiencing normoalbuminuria if the urine ENaC level shows a result of 0.98 ng/mL. The accuracy value is 72.4%, meaning that the accuracy of urine ENaC levels in predicting the occurrence of albuminuria (diabetic nephropathy) in T2DM with hypertension is 72.4%.

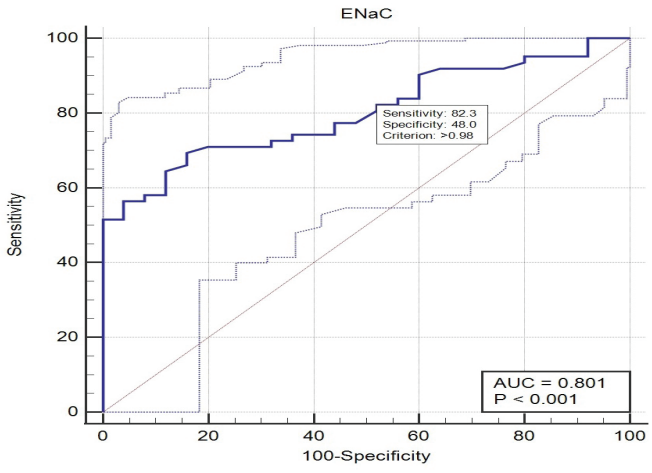

Figure Urine ENaC level

Table 1 Characteristics of Research Subjects

Chateristics	T2DM Groups		P Value
	Albuminuria	Normoalbuminuria	
	n=62	n=25	
Age (years)			0.025[b]*
Mean±SD	56±13	63±10	
Minimum-maximum	28–82	42–80	
Sex			0.061[a]
Male	31 (63%)	18 (37%)	
Female	31 (82%)	7 (18%)	
Hypertension (mmHg)			0.167[b]
Hypertension stage 1: (Sistolic 140–159 atau dyastolic 80–99)	51 (68%)	24 (32%)	
Hypertension stage 2: (Sistolic ≥160 atau dyastolic ≥100)	11 (92%)	1 (8%)	
Duration of DM			0.455[a]
≤10 years	47 (73%)	17 (27%)	
>10 years	15 (65%)	8 (35%)	
Fasting blood glucose (mg/dL)			0.350[b]
Achieving therapeutic targets (80–130 mg/dL)	9 (60%)	6 (40%)	
Not achieving therapeutic target (>130 mg/dL)	53 (74%)	19 (26%)	
eGFR (mL/min/1,73 m^2)			
Median	79.5	76	0.711[d]
Minimum-maximum	60–137	60–116	
ENaC (ng/mL)			<0.001[c]*
Mean±SD	1.90±0.874	0.99±0.438	
Minimum-maximum	0.38–3.93	0.21–1.85	

a:Chi-Square test; b:Fisher Exact test, c: unpaired t test, d: Mann Whitney test. *means p <0.05; significant or statistically significant T2DM: Type 2 diabetes mellitus; DM: Diabetes mellitus; eGFR: estimated glomerular filtration rate; ENaC: ephitelial sodium channel

Table 2 Sensitivity and Specificity Value of Urine ENaC Level from Various Cut-off Value

ENaC Cut-off Value (ng/mL)	Sensitivity (%)	Spesificity (%)	Positive predictive value (%)	Negative predictive value (%)
>0.21	100	8	72.9	100
>0.82	90.3	40	78.9	62.5
>0.88	83.9	40	77.6	50
>0.98	**82.3**	**48**	**79.7**	**52.2**
>1.28	69.4	84	91.5	52.5
>1.85	51.6	100	100	45.5

ENaC: ephitelial sodium channel

Table 3 The Validity of Urine ENaC Level Examination with uACR Gold Standard

`	T2DM Groups	
	Albuminuria	Normoalbuminuria
	n=62	n=25
ENaC (ng/mL)		
>0.98	51	13
≤0.98	11	12

Note: Sensitivity= a/(a+c)=51/62x100%= 82.3%; Specificity = d/(b + d)=12/25x100%=48%; Positive predictive value = a/(a + b)=51/64x100%=79.7%; Negative predictive value = d/(c + d)=12/23x100%= 52.2%; Accuracy= (a+d)/N=63/87x100%=72.4%; ENaC: ephitelial sodium channel; T2DM: type 2 diabetes mellitus

Discussion

In this study, subjects with albuminuria had a mean age ± 56 years old with DM occur most commonly at ≤10 years (73%) and 53 subjects (74%) of 62 subjects experienced an increase in fasting blood glucose level >130 mg/dL. The study by Shamshirgaran, et al[10] in Iran stated that DM patients aged <60 years who had poor glycemic control were more likely to experience DM complications, and patients with DM >7 years would experience DM complications 6 times faster than at the time they were diagnosed. Research Fasil, et al[11] in Ethiopia said that the prevalence of poor glycemic control in diabetic patients increased by 60.5% with a fasting blood glucose level.In this study that subjects with albuminuria had a younger age with DM duration of DM at <10 years and high glucose levels. This indicates that poor glycemic control can accerelate the occurance of kidney damage characterized by albuminuria. Therefore, The American Diabetes Association (ADA) recommends for T2DM patients to perform urine albumin examination starting when the patient is diagnosed with T2DM and carry out regular examinations every year.[12]

On the characteristics of blood pressure, subjects in the albuminuria and normoalbuminuria groups had uncontrolled hypertension. Based on research by Lastra et al[13] in the United States, hypertension occurs >50% in T2DM patients and contributes to the development of diabetic nephropathy and increases mortality compared to normotensive T2DM patients.Therefore, KDIGO recommends consistently lowering blood pressure in patients with T2DM < 140/90 mmHg so as to reduce the risk of cardiovascular disease and progressively reduce kidney injury.[14]

In this study, it was found that there was a significant difference in urine ENaC levels, namely the level was 2-fold increased in the albuminuria group (1.99 ng/mL). Hyperglycemia and hypertension in T2DM will stimulate ENaC mRNA transcription resulting in an increase in ENaC activity on the cell surface which causes increased sodium reabsorption from the tubules, sodium retention, aggravates hypertension, and has an impact on podocyte injury and kidney apoptosis.[2] Zheng et al[15] stated that increased proteinuria from plasmin, prostacyn and urokinase will activate proteolytics of the ENaC subunit which contributes to impaired renal sodium excretion and causes sodium retention, hypertension, and kidney injury. Increased damage to glomerular podocytes leads to leakage of proteases from the glomerulus into the tubular fluid causing increased ENaC activation leading to increased sodium retention and blood pressure.

In Table 3, it showed that in subjects with uACR results <30 mg/g (normoalbuminuria) there were 13 subjects (52%) who experienced an increase in urine ENaC levels >0.98 ng/mL. Based on these data, it shows that the examination of urine ENaC levels at a cut-off 0.98 ng/mL is close to the average normoalbuminuria group with a high sensitivity of 82.3% so that it is more ideally used as an examination to screen for the occurrence of diabetic nephropathy in T2DM patients with hypertension, especially patients with normoalbuminuria thereby reducing false negative results.

There is a limitation in this study, namely that the research subjects were not tested for HbA1C to determine the quality of glycemic control. In addition, the research subjects only had one examination of urine albumin levels which should be repeated at least 2 out of 3 examinations within a period of 3–6 months to determine the diagnosis of diabetic nephropathy.

In conclusion, the validity of urine ENaC level examination at a cut-off of 0.98 ng/mL with a sensitivity of 82.3%, it shows that the examination of urine ENaC levels in T2DM patients with hypertension can be used as a screening test for diabetic nephropathy. Examination of urine ENaC levels can be used as an alternative to uACR examination to screen for diabetic nephropathy,

especially in T2DM patients with hypertension without albuminuria.

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