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Analysis of Urinary Albumin and Urinary Synaptopodin Levels in Type 2 Diabetes Mellitus Subjects

***Afni Juhairia Laisouw[1,7], Liong Boy Kurniawan[2,5], Yuyun Widaningsih[2,5], Tenri Esa[2], Himawan Sanusi[3], Andi Alfian Zainuddin[4], Theosobia Grace Orno[6]**

[1]Master of Biomedical Sciences, Graduate School, Hasanuddin University Makassar, Indonesia. [2]Department of Clinical Pathology, Faculty of Medicine, Hasanudin University Makassar, Indonesia. [3]Department of Internal Medicine, Faculty of Medicine, Hasanudin University Makassar, Indonesia. [4]Department of Public Health and Community Medicine, Faculty of Medicine, Hasanudin University Makassar, Indonesia. [5]Hasanuddin University Hospital Makassar, Indonesia. [6]Medical Laboratory Technology Faculty, Health Polytechnic Ministry of Health Kendari, Indonesia. [7]Medical Laboratory Technology Faculty, Health Polytechnic Ministry of Health Maluku, Indonesia. *Email: ria.laisow@gmail.com
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Abstract: Hyperglycemia from diabetes mellitus affects many body organs and interferes with normal function. Kidney function decreased in approximately one-third of patients with diabetes mellitus before the development of albuminuria. The purpose of this study was to analyze differences in urine albumin and urine synaptopodin levels in type 2 diabetes mellitus (T2DM) and non-DM subjects, differences in urine synaptopodin levels in T2DM subjects with and without nephropathy, to analyze the correlation between urine albumin and urine synaptopodin in T2DM subjects and the cut-off analysis of sensitivity and specificity of urinary synaptopodin in diagnosing diabetic nephropathy. A sample of 60 subjects comprised 40 T2DM subjects and 20 non-DM subjects. Urinary synaptopodin levels were examined using the ELISA method, and albuminuria levels using the immunoturbidimetric method. Based on statistical analysis, the results showed that there were differences in urine albumin levels in T2DM and non-DM subjects (p*= < 0.001), there were differences in urine synaptopodin levels in T2DM and non-DM subjects (p*= < 0.001), there were no differences in urine synaptopodin levels with and without nephropathy in T2DM subjects (p*= 0.090), a relationship was found between urine albumin and urinary synaptopodin in T2DM subjects (p*= 0.048, r= 0.314) and the cut off of urinary synaptopodin in diagnosing nephropathy was ≥0.39 ng/mL, sensitivity 64.7% and specificity 56.5%. We recommend further prospective studies with larger sample sizes to compare urinary synaptopodin levels and microalbuminuria (MAU) as markers for early detection of DN in T2DM subjects.
Keywords: Urinary albumin; urinary synaptopodin; type 2 diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a health problem faced by all countries. This is caused by increased blood glucose levels originating from the inability of cells (beta) in the pancreas to produce sufficient insulin or due to the ineffective utilization of insulin by cells in the body (Berbudi et al., 2019).

The World Health Organization (WHO) report on diabetes in 2019 is predicted would increase to 693 million in 2045 (Artasensi et al., 2020). Based on the Pusdatin

Corresponding Author: Afni Juhairia Laisouw
Medical Laboratory Technology Faculty, Health Polytechnic Ministry of Health Maluku, Indonesia.
Email: ria.laisow@gmail.com

Ministry of Health (2018), the estimated number of people with diabetes mellitus in Indonesia in 2000 was 8.4 million people, and it is predicted to increase in 2030 to 21.3 million people, making Indonesia fourth in the world after the United States (Pusdatin Kemkes, 2018). The main classification of diabetes mellitus is divided into two types, namely type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (Care in Diabetes, 2021). Approximately 90% of cases of diabetes mellitus are T2DM, with characteristics of impaired insulin sensitivity or impaired insulin secretion. Clinically, DMT2 appears when the body can no longer produce enough insulin to compensate for increasing insulin resistance (Decroli, E, 2019).

Hyperglycemia from diabetes mellitus affects many body organs and interferes with normal function (Galicia et al., 2020). Structural and functional disorders of the vascular organ system cause micro and macrovascular complications. These complications affect the organs of the body, including, in particular, the eyes, kidneys, heart and nerves (Banday et al., 2020). One of the microvascular complications related to the kidney is diabetic nephropathy (Politano et al., 2020).

Diabetic nephropathy (DN) caused by diabetes mellitus is one of the major causes of end-stage kidney failure worldwide, often associated with T2DM (Davies et al., 2022). The increasing incidence of T2DM increases the frequency of diabetic nephropathy (Zhang et al., 2020). Diabetic nephropathy is characterized by albuminuria, interference glomerular filtration rate (GFR) or both (Sloan, 2019). In general, the appearance of microalbuminuria has been used to detect the presence of diabetic nephropathy. Podocyturia is an earlier marker of DN than albuminuria (Mukherjee, 2018) because kidney function decrease in about one-third of patients before the appearance of albuminuria, which makes it inadequate to monitor the occurrence and development of DN (Zhang et al., 2018). Injured podocytes can cause decreased levels of podocyte-specific proteins (Sanchez-Niño et al., 2019).

Synaptopodin is a protein that regulates the actin cytoskeleton (Feng et al., 2020). The actin cytoskeleton acts as the backbone of the podocyte because the proteins that regulate or stabilize the actin cytoskeleton contribute to normal podocyte function. Changes in actin or actin regulatory proteins will lead to changes in the shape and function of podocytes (Samsu. N, 2018). Urinary podocytes and their specific proteins can be considered biomarkers in podocyte injury due to a decrease in the number of podocytes and loss of the foot process (FP), which often occurs in the early stages of DN due to the flattening of podocytes. Urinary podocytes are challenging to measure directly, so podocyte-specific protein products are used to detect them (Zhang et al., 2018).

One study by Wang et al. (2007) showed that synaptopodin urine mRNA levels in DN patients were very high compared to control subjects using the PCR method. These results were also proven by a kidney biopsy which stated synaptopodin levels had a positive correlation with urinary albumin excretion (J. Zhang et al., 2018); this was different from the study of Kwon, K.S. et al. (2016), which stated that urine synaptopodin levels could predict diabetic kidney disease independently of urinary albumin excretion. Previous studies have focused on diagnosing synaptopodin as a biomarker of diabetic nephropathy without assessing synaptopodin in T2DM subjects in general, whereas, in theory, chronic hyperglycemia can reduce kidney function. Therefore, this cross-sectional study aims to was to analyze differences in urine albumin and urine synaptopodin levels in (T2DM and non-DM subjects, differences in urine synaptopodin levels in T2DM subjects with and without DN, to analyze the correlation between urine albumin and

urine synaptopodin in T2DM subjects and to analyze the cut-off of sensitivity and specificity of urinary synaptopodin in diagnosing diabetic nephropathy.

MATERIALS AND METHODS

This research was an observational study with a case-control research design. This research received ethical approval from the Health Research Ethics Commission (KEPK) Hasanuddin University Faculty of Medical-UNHAS (RSPTN UH) with ethical number 665/UN4.6.4.5.31/PP36/2022.

Sampling locations were carried out at the Endocrine Polyclinic at Hasanuddin University Hospital, the Clinical Pathology Laboratory at the Hospital of Hasanuddin and the Clinical Pathology Laboratory at RSUP. Wahidin Sudirohusodo Makassar. Furthermore, the research was conducted at the Hasanuddin University Medical Research Center (HUM-RC) Laboratory, Hasanuddin University Makassar State University Hospital and the Parahita Makassar Clinical Laboratory. 60 subjects were grouped into T2DM and non-DM groups using a non-probability sample technique. The inclusion criteria in this study were subjects willing to participate in a series of studies, subjects with T2DM and non-DM. They excluded if they had kidney disease not caused by diabetes mellitus. The diagnosis of DM subjects was based on the results of laboratory tests, namely fasting blood glucose levels of 126 mg/dL or HbA1c values of 6.5%. Diagnosis of DN in T2DM subjects by examining the urine albumin creatinine ratio (ACR) with an interpretation of the results of <30 mg/g means not diagnosed with diabetic nephropathy and if ≥30 mg/g means diagnosed with diabetic nephropathy.

In sample preparation, we used 10 mL of urine in the middle portion, divided into two parts for measurement of urinary albumin and urinary synaptopodin. Each tube was centrifuged at 3000 rpm for 20 minutes. Pipette 300 µL of the urine supernatant obtained into the sample cup for testing immediately or store the sample at -20^{0}C. The measurement of urine synaptopodin levels used the Enzyme-linked Immunosorbent Assay (ELISA) method with Assay Genie® at a wavelength of 450 nm and reported in ng/mL. The measurement of urine albumin levels used the Cobas 311 spectrophotometer instrument with the immunoturbidimetric method and reported in mg/g.

Data processing was performed using the SPSS 25 computer program. The data analysis used was the Shapiro-Wilk normality test, the Mann-Whitney test because the data were not normally distributed, Spearman's test and Receiver Operating Characteristic (ROC) analysis. Significant results if p* <0.05.

RESULTS AND DISCUSSION

Table 1 shows that gender characteristics of the theT2DM research subjects obtained more female; there are 24 subjects (60%), while 16 subjects (40%) were male, with a total of 40 subjects (100%) and non-DM subjects obtained the type the male sex was more numerous, namely 12 subjects (60%). In comparison, women were eight subjects (40%) with 20 subjects (100%). Age characteristics of T2DM subjects show that the most recent elderly group (56-65 years) are 20 subjects (50%), the early elderly group (46-55 years) is 12 subjects 30%), the late adult group (36 -45 years) as many as six subjects (15%) and the elderly group (≥65 years) as many as two subjects (5%) and non-DM subjects show the latest adulthood group (36-45 years), namely ten subjects (50%), the early adulthood group (18-35 years) as many as nine subjects (45%), and the elderly group (65 years) as many as one subjects (5%). The characteristics of urine albumin levels (mg/g) of T2DM subjects

show the category of normoalbuminuria (<30 mg/g) in 23 subjects (57.5%), microalbuminuria (30-300 mg/g), namely ten subjects (25%) and macroalbuminuria (≥300 mg/g) in 7 subjects (17.5%). In contrast, in non-DM subjects, the characteristics of urine albumin levels (mg/g) showed 20 subjects (100%) included in the normoalbuminuria category (<30 mg/g).

Table 1. Subjects Characteristics

Characteristics	N	%
T2DM subjects (n= 40)		
Gender		
Male	16	40
Female	24	60
Age (years)		
The latest adulthood (36-45)	6	15
Early elderly (46- 55)	12	30
Most recent elderly (56- 65)	20	50
Elderly (≥ 65)	2	5
Urine Albumin Levels (mg/g)		
Normoalbuminuria (< 30 mg/g)	23	57.5
Microalbuminuria (30 – 300 mg/g)	10	25
Macroalbuminuria (≥ 300 mg/g)	7	17.5
Non-DM subjects (n=20)		
Gender		
Male	12	60
Female	8	40
Age (years)		
Early adulthood (18-35)	9	45
The latest adulthood (36-45)	10	50
Early elderly (46- 55)	1	5
Urine Albumin Levels (mg/g)		
Normoalbuminuria (< 30 mg/g)	20	100

Table 2. Differences in Urinary Albumin and Urinary Synaptopodin Between Groups

Variables	Subjects	Mean	SD	*p*-value*
Urinary albumin (mg/g)	T2DM	374.38	1301.69	<0.001
	Non-DM	4.85	3.01	
Urinary synaptopodin (ng/mL)	T2DM	0.58	0.61	<0.001
	Non-DM	0.28	0.13	

*Mean difference test was conducted using the Mann-Whitney test

Table 2 shows the study results found that the average urinary albumin level of the T2DM group (374.38 mg/g) was higher than non-DM (4.85 mg/g), and statistical tests showed that there was a difference between urinary albumin levels in T2DM and non-DM subjects. This is because, in T2DM subjects, there is improvement in blood glucose levels due to abnormalities in insulin secretion, insulin action or both in T2DM subjects, causing dysfunction of glomerular and renal tubular filtration resulting in increased excretion albumin. Albuminuria is a sign of kidney disease with much albumin in the urine. Microalbuminuria is a biomarker in T2DM as a test for diabetic nephropathy (Gluhovschi et al., 2016).

The mean urinary synaptopodin level of the T2DM group (0.58 ng/mL) was higher than non-DM (0.28 ng/mL), and statistical tests showed that there was a difference between urinary synaptopodin levels in T2DM subjects and non-DM (p*<0.05). The glomerulus is a structure that supports physiological functions and the collaborative interaction of several cell types: endothelial cells, mesangial cells, podocyte cells, and the glomerular basement membrane (MBG). Podocytes are one of the cells in the glomerulus which play an essential role in the pathogenesis of glomerular diseases. Podocyte injury can cause the release of molecules originating from podocytes into the urinary space, including synaptopodin (Jana et al., 2022). This is in line with a study by Wang et al., which showed that the level of urinary synaptopodin in diabetes patients was higher than that found in control subjects (Wang et al., 2007) and a study by Hara et., 2012 al which stated that urine synaptopodin in patients diabetes mellitus type 2 has higher levels than control samples even before the occurrence of proteinuria (Hara et al., 2012).

Table 3. Differences in Urinary Synaptopodin in T2DM Subjects With and Without Nephropathy

Variable	T2DM Subjects	Mean	SD	*p*-value*
Urinary synaptopodin (ng/mL)	With nephropathy	0.69	0.77	0.090
	Without nephropathy	0.50	0.47	

*Mean difference test was conducted using the Mann-Whitney test

Based on Table 3, the study's results found that the average urinary synaptopodin level of the T2DM group with nephropathy (0.69 ng/mL) was higher than T2DM without nephropathy (0.50 ng/mL). However, statistical tests showed no difference between urinary synaptopodin levels in T2DM subjects with and without nephropathy (p* >0.05). İt caused by high blood glucose levels in T2DM patients have affected the kidneys, and kidney function has decreased in about a third of diabetes mellitus patients before albuminuria occurs, so in T2DM patients, screening must be started when diabetes mellitus is initially diagnosed because 7% of patients already had microalbuminuria at the time of diagnosis. Recent studies have shown that podocyte-specific proteins such as synaptopodin are present in the urine of diabetic patients before the appearance of albuminuria. Synaptopodin is a protein that can describe the condition of podocytes in urine (Decroli. E., 2019) because it regulates the actin cytoskeleton (Feng e at, 2020). The actin cytoskeleton is the backbone of the podocyte, which contributes to normal podocyte function (Samsu. N., 2018). Injury to the podocytes causes synaptopodin to be present in the urine.

Table 4. Correlation Between Urinary Albumin and Urinary Synaptopodin in T2DM Subjects

		Urinary synaptopodin (ng/mL)
Urinary albumin (mg/g)	R	0.314
	P	0.048
	N	40

*Spearman-rho test, r= correlation, p= level of significance, n= number of samples

Table 4 shows a correlation between urinary albumin and synaptopodin in T2DM subjects (p=0.0<0.05; r=0.314). Based on the level of correlation strength, this value shows that the level of correlation strength is sufficient and has a positive relationship between the two variables. This study's results align with the research of J. Zhang et al. (2018), which stated that synaptopodin levels were positively correlated with urinary albumin excretion and research from Chen. Z et al. (2019) concluded that urine synaptopodin positively correlated with 24-hour urine protein. Research by Wang et al. (2007) found a correlation between urine albumin and synaptopodin. This follows the theory that states that albuminuria in diabetes occurs due to glomerular dysfunction. The glomerulus is a structure that supports physiological functions and the collaborative interaction of several cell types: endothelial cells, mesangial cells, podocyte cells, and the glomerular basement membrane (MBG).

Podocytes are one of the cells in the glomerulus that play an essential role in the pathogenesis of glomerular diseases (Samsun, 2018). Podocyte injury causes a decrease in the number of podocytes and loss of the foot process (FP), which causes thickening of the MBG, which often occurs in the early stages of diabetic nephropathy (DN) due to apoptosis or FP flattening so that urine podocytes and their specific protein products can be found in the urine (J. Zhang et al., 2018). Synaptopodin is one of the proteins that can describe the condition of podocytes in urine (Decroli. E., 2019) because it regulates the actin cytoskeleton (Feng e at, 2020). The actin cytoskeleton is the backbone of the podocyte, which contributes to normal podocyte function (Samsu. N, 2018). Synaptopodin levels decrease in DN patients because high and uncontrolled blood glucose levels suppress synaptopodin expression in podocytes (Sanchez-Niño et al., 2019).

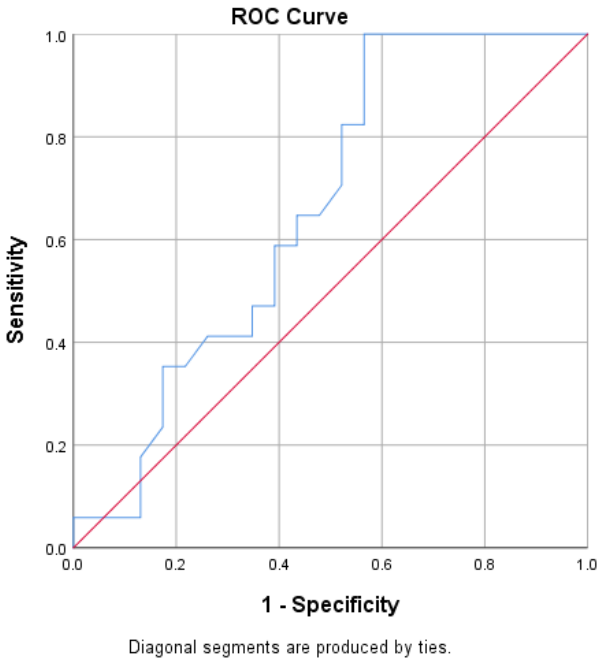

Figure 1. Receiver Operating Characteristic (ROC) Curve of Urinary Synaptopodin

The area under the ROC curve value obtained was 65.9%, which means that if the urinary synaptopodin value were used to diagnose diabetic nephropathy in 100 T2DM subjects, then the correct conclusion would be obtained in 66 people. The

sensitivity of a biomarker is determined by several factors, including the number of samples used in the assay and the specific characteristics of the sample. In this study, the sensitivity obtained was 65.9% due to the disproportionate sample size between groups.

Table 5. Sensitivity, Specificity and Cut-off of Urinary Synaptopodin

Coordinate points	Cut Off	Sensitivity	Specificity
14*	0.39*	0.647	0.565

Table 5 shows the recommended cut-off of urinary synaptopodin in diagnosing nephropathy at point 14, which is $\geq$ 0.39 with a sensitivity of 64.7% and a specificity of 56.5%. The limitations of this study were only seeing the relationship between albuminuria and urinary synaptopodin in T2DM subjects, without looking at the relationship between urinary synaptopodin and each sub-group of albuminuria in T2DM subjects and the number of samples in this study was small.

CONCLUSION

The results of this study concluded that there was a difference in urinary albumin levels in T2DM and non-DM subjects, found differences in urinary synaptopodin levels in T2DM and non-DM subjects, no differences between urinary synaptopodin levels in T2DM subjects with and without diabetic nephropathy, a significant correlation between urinary albumin and urinary synaptopodin in T2DM subjects and. The results of this study recommend the use of synaptopodin urine cut-off is ≥0.39 with a sensitivity of 64.7% and a specificity of 56.5%. We recommend further prospective studies with larger sample sizes to compare urinary synaptopodin levels and microalbuminuria (MAU) as markers for early detection of DN in T2DM subjects.

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CONFLICT OF INTEREST

The authors have no potential conflicts of interest regarding this study.

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