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COMPARATIVE ANALYSIS OF URINARY NEPHRIN AND SERUM NEPHRIN AS EARLY MARKERS OF DIABETIC NEPHROPATHY IN TYPE 2 DM

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Abstract

Diabetes mellitus is a chronic disease whose progressiveness can lead to microvascular complications in the form of diabetic nephropathy. Early markers are needed to prevent end stage renal disease (ESRD), one of which is nephrin which is the main component of slit diaphragm. The purpose of this study was to analyze the comparison of urine nephrin and serum nephrin levels as early markers of diabetic nephropathy in type 2 DM. The cross sectional study used samples of type 2 DM who were treated at Hasanuddin University Hospital and examined for urine nephrin, serum nephrin and urine albumin levels. The sample group consisted of type 2 DM patients with normoalbuminuria and microalbuminuria. Statistical test with Mann Whitney test, Chi-Square, Kolmogorov smirnov and ROC curve. Serum nephrin and urinary nephrin levels in type 2 DM patients with microalbuminuria were higher than those in type 2 DM patients with normoalbuminuria (p = <0.001). There was a significant difference between serum nephrin and urine nephrin levels in the type 2 DM group with normoalbuminuria (p=0.002), but there was no significant difference in the microalbuminuria group (p=0.485). Examination of serum nephrin and urine nephrin levels can be used as early markers of diabetic nephropathy in type 2 DM with cut-off values for serum nephrin and urine nephrin are 3.09 ng/mL and 6.84 ng/mL with sensitivity and specificity for serum nephrin are 96% and 60% while sensitivity and specificity for urine nephrin are 92% and 73.3%.

Keywords: Urinary Nephrin, Serum Nephrin, Type 2 DM, Diabetic Nephropathy.

INTRODUCTION

Uncontrolled diabetes mellitus will lead to various chronic complications, both microvascular and macrovascular. Manifestations of macrovascular complications can include coronary heart disease, cerebral thrombosis and gangrene. Diseases due to microvascular complications that can occur in diabetic patients are retinopathy, neuropathy and diabetic nephropathy [1].

Diabetic nephropathy (ND) is a microvascular complication of diabetes mellitus characterized by decreased glomerular filtration rate (LFG) and albumin in the urine (albuminuria). Diabetic nephropathy is one of the major microvascular complications of DM, contributing to increased morbidity and mortality [2]. Diabetic nephropathy is the leading cause of chronic kidney disease (CKD) and about 40% of end-stage-renal disease (ESRD) worldwide [3].

The average incidence of diabetic nephropathy is high (about 3% per year) after 10 to 20 years of diabetes onset. One study found the incidence of diabetic nephropathy to be higher than the incidence of other microvascular complications such as retinopathy and neuropathy [4]. The progression of the disease occurs in stages and is associated with glycemic control and blood pressure. It is estimated that more than 20% to 40% of diabetic patients will progress to chronic kidney disease (CKD) depending on the population with a significant number progressing to ESRD requiring renal replacement therapy such as kidney transplantation [5]. This therapy has a major economic impact

on patients and their families. Early detection of renal involvement in patients with type 2 DM is important for effective and efficient treatment and slowing disease progression to ESRD [6].

Diagnosing DM patients leading to ND process is very important. So far microalbuminuria is considered the gold standard in early detection of ND although it is a non-specific marker that can simultaneously be present in other pathological conditions such as urinary tract infection, cardiovascular disease, in non-diabetic patients and others. Albuminuria has been one of the biomarkers used to screen renal function and generally reflects glomerular injury and increased glomerular permeability to macromolecules, but may not be detected in the early stages [6].

Diabetic nephropathy causes pathological changes in the structural and functional components of the kidney due to glomerular and tubular injury resulting in hyperfiltration which results in albuminuria. The pathogenesis of ND begins with chronic hyperglycemia that activates metabolic pathways, increased production of proinflammatory cytokines and activation of hemodynamic pathways. Abnormalities in the glomerulus can be found in the basement membrane, podocytes and capillary endothelium. There are three components that form the glomerular filtration barrier: podocytes, capillary endothelial cells and the glomerular basement membrane. Several proteins that describe the condition of podocytes, such as urinary nephrin, synaptopodin, podocalixyn and podocin have increased expression in ND patients [7].

Human biopsy studies have shown that both functional and structural podocyte injury occurs at a very early phase of ND. Podocytes in the glomerulus will also transition into podocytes that will cause podocyte dysfunction and detachment, this process is called epithelial membrane transition (EMT). The activity of EMT causes podocyte damage, decreased podocyte density, podocyte apoptosis and podocyte shedding. These processes will cause changes in foot processes in the podocyte diaphragm slit so that albuminuria will occur [7]. Koziolek, M. et al., 2020, found that nephrinuria was found in 100% of patients with type 2 DM and macroalbuminuria, in 88% with microalbuminuria, and 82% of patients with type 2 DM and normoalbuminuria [8] .

Based on this background and the lack of research comparing serum nephrin and urine nephrin levels as early markers of diabetic nephropathy in patients with type 2 DM, the researcher is interested in researching with the aim of analyzing the comparison of urine nephrin and serum nephrin levels as early markers of diabetic nephropathy in type 2 DM which can be considered by clinicians as early markers in predicting diabetic nephropathy complications in patients with type 2 DM and can provide earlier treatment as a form of prevention towards end stage renal diase (ESRD).

RESEARCH METHODS

This study used a cross sectional design by taking a sample of 45 people from patients with type 2 DM suspected of diabetic nephropathy who sought treatment at the endocrine clinic of Hasanuddin University Hospital Makassar. The research sample is an affordable population that meets the inclusion criteria, namely type 2 DM patients aged ≥ 18 years who do not have a history of kidney disease that is not a complication of type 2 DM and cardiovascular disease, urinary tract infections and malignancies. Patients were further classified based on their urine albumin levels, namely type 2 DM with normoalbuminuria and microalbuminuria.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 22. Statistical analysis methods used were descriptive statistical calculations, Mann Whitney statistical test, Chi Square, and Receiver Operating Characteristics (ROC) curve statistical test to obtain cut off values and see the ability of early markers of serum nephrin and urine nephrin. Test results were considered significant if $p < 0.05$. Ethical approval was obtained from the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Hasanuddin University National Hospital.

RESULT

The results showed that of the 40 samples of type 2 DM patients there were 19 (47.5%) men and 21 (52.5%) women with an age range of 38 - 69 years. The sample of type 2 DM patients consisted of 3 (7.5%) controlled DM and 37 (92.5%) uncontrolled DM, 27 (67.5%) obesity and 13 (32.5%) non-obesity, 20 (50%) hypertension and 20 (50%) non-hypertension. The median values of the biochemical parameters of type 2 DM patients were GDP 162 mg/dL, HbA1c 10.2%, creatinine 0.9 mg/dL, eGFR 85.5 ml/min/1.73 m2, and UACR 60 mg/dL (Table 1).

Table 1: Characteristics of the research sample

Criteria	Median (Min-Max)	n (%)
Gender		
Male		19 (47,5)
Female		21 (52,5)
Age (Years)	54 (38 - 69)	
GDP (mg/dL)	162 (80 - 449)	
HbA1c (%)	10,2 (5,6 - 17)	
Creatinine (mg/dL)	0,9 (0,3 - 6,6)	
eGFR (ml/min/1,73 m2)	85,5 (9 - 140)	
UACR (mg/dL)	60 (4 - 4700)	
DM duration		
≤ 5 Years		16 (40)
6 – 10 Years		7 (17,5)
> 10 Years		17 (42,5)
DM Controlled		
Controlled		3 (7,5)
Uncontrolled		37 (92,5)
UACR Classification		
Normoalbuminuria		15 (37,5)
Mikroalbuminuria		25 (62,5)
Obesity		
Non-obesity		13 (32,5)
Obesity		27 (67,5)
Hypertension		
Non-HT		20 (50)
HT		20 (50)
BMI (Kg/m^2)	26,3 (19,3 - 40,0)	
Serum Nephrin Level (ng/mL)	4,78 (1,17 - 20,11)	
Urine Nephrin Level (ng/mL)	7,33 (3,91 - 11,07)	

Tabel 2: Comparison of anthropometric, biochemical, and urinary and serum nephrin data between type 2 DM patients with normoalbuminuria and microalbuminuria

Variable	Normoalbuminuria	Mikroalbuminuria	P
Age (Years)	55 (39 – 69)	53 (38 – 68)	0,956
BMI (Kg/m^2)	26 (22,9 – 34,9)	28,3 (19,3 – 40,9)	0,391
GDP (mg/dL)	148 (107 – 272)	186 (80 – 449)	0,065
HbA1c (%)	8,6 (5,6 – 12,6)	11 (6,4 – 17)	0,088
Creatinine (mg/dL)	0,7 (0,4 – 1,3)	1 (0,3 – 6,6)	0,013
eGFR (ml/menit/1,73 m2)	104 (59 – 129)	75 (9 – 140)	0,005
UACR (mg/dL)	10 (4 – 30)	287 (32 – 4700)	< 0,001
Serum Nephrin Level (mg/dL)	2,96 (1,17 – 8,74)	6,84 (3,06 – 20,11)	< 0,001
Urine Nephrin Level (mg/dL	6,10 (3,91 – 9,25)	7,63 (6,29 – 11,1)	< 0,001

In this study, the samples of type 2 DM patients with normoalbuminuria were 15 people (37.5%) and microalbuminuria were 25 people (62.5%). Based on Table 2 through the Mann Whitney analysis test, there was a significant difference between the two groups of type 2 DM based on UACR levels in creatinine levels, eGFR, UACR levels, serum nephrin levels and urine nephrin levels with p values of 0.013; 0.005; < 0.001; < 0.001; and < 0.001 ($p < 0.05$), respectively.

Table 3: Comparison of risk factors for diabetic nephropathy between type 2 DM patients with normoalbuminuria and microalbuminuria

Variable	Normoalbuminuria	Mikroalbuminuria	P
DM duration			0,066*
≤ 5 Years	10	6	
6 – 10 Years	0	7	
> 10 Years	5	12	
DM Controlled			0,545**
Controlled	2	1	
Uncontrolled	13	24	
Obesity			0,433***
Non-obesity	6	7	
Obesity	9	18	
Hypertension			0,327***
Non- Hypertension	9	11	
Hypertension	6	14	

*** Kolmogorov-smirnov test**

****Fisher exact test**

***** Chi square test**

Table 4. Comparison of serum nephrin and urine nephrin levels based on UACR level classification

Variable	Serum Nephrin (ng/mL)	Nephrin Urine (ng/mL)	P
Normoalbuminuria	2,96 (1,17 – 8,74)	6,10 (3,91 – 9,25)	0,002
Mikroalbuminuria	6,84 (3,06 – 20,11)	7,63 (6,29 – 11,1)	0,485

Based on table 4, the median urinary nephrin level in the normoalbuminuria group was higher at 6.10 ng/mL compared to the serum nephrin level of 2.96 ng/mL and the median urinary nephrin level in the microalbuminuria group was slightly higher at 7.63 ng/mL compared to the serum nephrin level of 6.84 ng/mL. Through the Mann Whitney

analysis test, there was a significant difference between serum nephrin and urine nephrin levels in the normoalbuminuria group with a P value of 0.002 ($p < 0.05$), while in the microalbuminuria group there was no significant difference with a p value of 0.485 ($P > 0.05$).

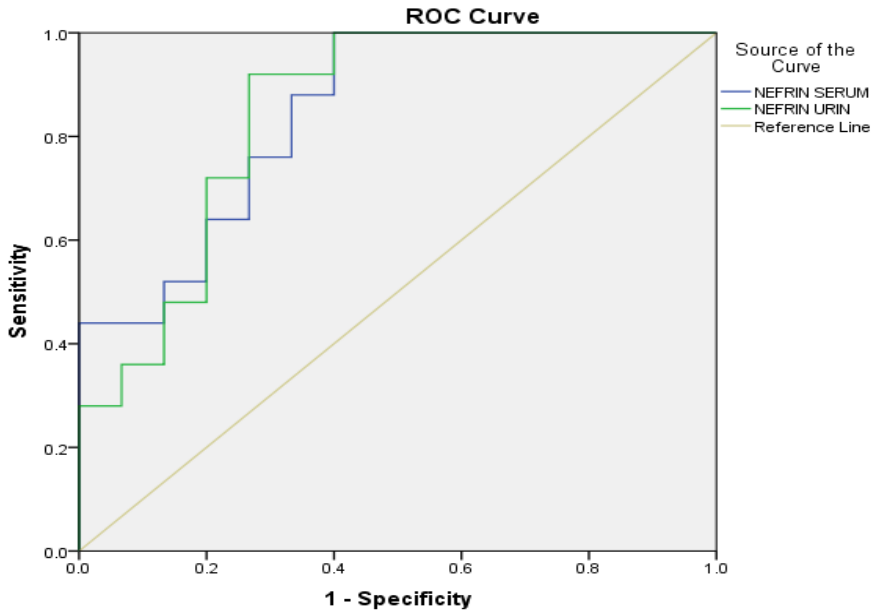

Figure 1: ROC analysis of differences in serum nephrin and urine nephrin levels in type 2 DM patients as early markers of diabetic nephropathy

Based on Figure 1, the area under the curve (AUC) of serum nephrin and urine nephrin were 0.845 ($p < 0.001$) and 0.845 ($p < 0.001$), indicating that serum nephrin and urine nephrin can be used as early markers of ND in type 2 DM. Based on the ROC curve coordinates, the cut-off values for serum nephrin and urine nephrin were 3.09 ng/mL and 6.84 ng/mL with sensitivity and specificity for serum nephrin were 96% and 60% while the sensitivity and specificity for urine nephrin were 92% and 73.3% (table 4).

Table 4: ROC analysis of differences in serum nephrin and urine nephrin levels in type 2 DM patients as early markers of diabetic nephropathy

Parameters	AUC	95% CI for AUC	Sensitivity (%) 95 % CI	Specificity (%) 95 % CI	p-Value
Serum nephrin	0,845	0,718-0,972	96	60	< 0,001
Nephrin urine	0,845	0,708-0,982	92	73,3	< 0,001

DISCUSSION

Diabetic nephropathy is one of the microvascular complications of type 2 DM which about 40% can progress to ESRD. Microalbuminuria was previously considered the gold standard for early detection of ND. Several studies have shown that microalbumin in urine is a nonspecific and nonsensitive marker for early detection of ND as it can also be elevated in other pathological conditions such as urinary tract infection, acute illness, cardiovascular disease. The presence of microalbumin in urine indicates damage to all three components of the glomerular filtration barrier i.e. endothelium, glomerular basement membrane and podocytes, and its diagnostic accuracy is limited by the fact that structural damage may precede microalbumin excretion in urine. The presence of nephrin and other podocyte-specific proteins in the urine suggests

podocyte damage, independent of both other components of the glomerular filtration barrier. Thus, it is suspected that there is podocyte damage before the appearance of microalbuminuria and proteinuria [9].

In this study, type 2 DM patients with microalbuminuria were found to be more than those with normoalbuminuria, this is related to more type 2 DM patients in this study who were more than 10 years old, accompanied by uncontrolled DM, hypertension and obesity. These results are in line with the research of Zaccardi, et al., 2018, which found the relationship between HbA1c, duration of DM, obesity, and hypertension with the incidence of albuminuria in patients with type 2 DM [10]. Hyperglycemia in diabetes mellitus leads to increased adiposity and inflammation, which predisposes to podocyte insulin resistance and glomerular dysfunction resulting in albuminuria. Albuminuria is associated with endothelial dysfunction. In 20-40% of type 2 DM, microalbuminuria begins within 10-15 years after DM onset, which within the next 5 years will result in macroalbuminuria. With risk factor control, patients with microalbuminuria have more chances of spontaneous return to normal. Complete remission is highly unlikely in patients with macroalbuminuria, but the progression leading to ND can be slowed [11].

UACR levels in the study were found to increase in type 2 DM patients with microalbuminuria and there was a significant difference between the two sample groups with a p-value <0.001. In patients with type 2 diabetes, hyperglycemia in the blood and hypoglycemia in the tissues trigger lipolysis and proteolysis which results in the production of reactive oxygen species (ROS) and advanced glycation end products (AGE). AGE receptor binding to AGEs will trigger downstream signaling that facilitates the generation of free radicals, activates inflammatory cells, increases the synthesis of angiotensin II, and the production of growth factors such as vascular, endothelial and TGF-β factors that eventually lead to proteinuria [9].

Serum nephrin and urinary nephrin levels were found to be higher in type 2 DM patients with microalbuminuria and there was a significant difference between the two sample groups. In this study, there was no significant difference between serum nephrin and urine nephrin levels. The cut-off values for serum nephrin and urine nephrin were 3.09 ng/mL and 6.84 ng/mL with the sensitivity and specificity for serum nephrin being 96% and 60% while the sensitivity and specificity for urine nephrin were 92% and 73.3%. This shows that serum nephrin and urine nephrin start to increase even before the onset of proteinuria. Nephrin is a better indicator of early kidney injury even in ND before the onset of albuminuria. Phosphorylation of tyrosine residues of nephrin mediates recovery of glomerular injury thereby maintaining glomerular function. Decreased nephrin expression as found in diabetes, impairs the ability of podocytes to recover after injury making them vulnerable to podocyte shedding. In patients with type 2 DM, approximately 35-57% of patients with CKD do not present with albuminuria. It has been found that nonalbuminuria CKD is associated with advanced glomerular lesions compared to patients with albuminuria CKD [12].

Nephrin is a podocyte-associated protein that plays a key role in maintaining the structure and functional integrity of the renal filtration barrier. Nephrin is expressed extending on the lateral aspect into the diaphragmatic cleft and is a major component of the diaphragmatic cleft and inhibits the filtration of large molecules into the urinary space without which glomerular function would be impaired leading to proteinuria [13]; [14]. The N-terminal domain of CD2 adaptor protein (CD2AP) can bind p85 and facilitate Nephrin-induced phosphoinositide 3-OH kinase (PI3K) pathway signaling,

which protects podocytes from apoptosis. In the glomerulus, CD2AP is also located in the diaphragm cleft and can interact with Nephrin via its C-terminal domain [15].

Wang et al conducted a study to investigate podocyte-related molecular expression genes in urine sediment from 21 patients with diabetic nephropathy and 9 healthy controls [18]. The researchers found that urinary nephrin levels (measured by mRNA expression) correlated with proteinuria (r=0.502, p=0.020) but not with eGFR. Nephrinuria was greater in diabetics with nephropathy, and the researchers concluded that nephrin measurements may play a role in clinical stratification of diabetic nephropathy patients. Nascimento et al used mRNA RT-PCR to measure urinary nephrin in a group of 15 controls and 67 diabetics. The study subjects were divided into 3 different groups: normoalbuminuria (NO) (<30 mg/g creatinine); microalbuminuria (MI) (30-300 mg/g creatinine); and macroalbuminuria (MA) (>300 mg/g creatinine). The researchers found that urine nephrin was higher in diabetics than non-diabetics and correlated with increased albuminuria. Nephrinuria was found in 53%, 71%, and 90% of NO, MI and MA diabetics respectively (p=0.023). The researchers concluded that diabetic subjects had increased urinary mRNA levels of podocyte proteins such as nephrin compared to non-diabetic subjects, even NO patients. In another study, Patari et al. showed the presence of nephrin fragments using immunohistochemistry and western blotting techniques in the urine of type 1 diabetics with or without nephropathy [16]. All previous studies are in line with the results obtained in this study.

The limitations of the study are the small number of samples, cross sectional research design so that it cannot directly see the relationship between nephrin levels both in serum and urine with albuminuria and diabetic nephropathy. It is hoped that future studies can use samples of diabetic nephropathy patients in all stages with a large sample size and use a better research design.

CONCLUSIONS

Serum nephrin and urinary nephrin levels in type 2 DM patients with microalbuminuria are higher than those in type 2 DM patients with normoalbuminuria. There is a significant difference between serum nephrin and urinary nephrin levels in the type 2 DM group with normoalbuminuria, but there is no significant difference in the microalbuminuria group. Examination of serum nephrin and urine nephrin levels can be used as an early marker of diabetic nephropathy in type 2 DM with cut-off values for serum nephrin and urine nephrin are 3.09 ng/mL and 6.84 ng/mL with sensitivity and specificity for serum nephrin are 96% and 60% while sensitivity and specificity for urine nephrin are 92% and 73.3%.

Reference

1) Harie Satria, Eva Decroli, A. Faktor Risiko Pasien Nefropati Diabetik Yang Dirawat Di Bagian Penyakit Dalam Rsup Dr. M. Djamil Padang. *J. Kesehat. Andalas* **7**, 149 (2018).

2) Mohamed, M. M., Eissa, S., Mostafa, M. & Hegazy, M. G. A. Diabetic Nephropathy Assessment: Microtubule-Associated Protein 1 Light-Chain 3B a New Promising Biomarker. *Indian J. Clin. Biochem.* **34**, 472–478 (2019).

3) Zhang, J., Liu, J. & Qin, X. Advances in early biomarkers of diabetic nephropathy. *Rev. Assoc. Med. Bras.* **64**, 85–92 (2018).

4) Saputri, R. D. Komplikasi Sistemik Pada Pasien Diabetes Melitus Tipe 2. *J. Ilm. Kesehat. Sandi Husada* **11**, 230–236 (2020).

5) Sulaiman, M. K. Diabetic nephropathy: Recent advances in pathophysiology and challenges in dietary management. *Diabetol. Metab. Syndr.* **11**, 1–5 (2019).

6) Kostovska, I. *et al.* Role of urinary podocalyxin in early diagnosis of diabetic nephropathy. *Rom. J. Intern. Med.* **58**, 233–241 (2020).

7) Benedictus, N. Nephrinuria Sebagai Faktor Risiko Nefropati Diabetik Pada Pasien Diabetes Melitus Tipe 2. (Universitas Brawijaya, 2014).

8) Koziolek, M. *et al.* Urine E-cadherin: A marker for early detection of kidney injury in diabetic patients. *J. Clin. Med.* **9**, (2020).

9) Veluri, G. & Mannangatti, M. Urinary Nephrin is a Sensitive Marker to Predict Early Onset of Nephropathy in Type 2 Diabetes Mellitus. *J. Lab. Physicians* (2022) doi:10.1055/s-0042-1757718.

10) Zaccardi, F., Davies, M. J., Griffin, S. J., Wareham, N. J. & Simmons, R. K. Cardiovascular risk factors and incident albuminuria in screen- detected type 2 diabetes. *Diabetes Metab Res Rev.* **33**, 1–12 (2018).

11) Kondapi, K., Kumar, N. L., Moorthy, S. & Silambanan, S. A study of association of urinary nephrin with albuminuria in patients with diabetic nephropathy. *Indian J. Nephrol.* **31**, 142–148 (2021).

12) Kishore, K., Silambanan, S., Moorthy, S. & Moorthy, S. Diagnostic sensitivity of biomarkers in assessing the occurrence and progression of diabetic nephropathy. *Eur. J. Mol. Clin. Med.* **8**, 1554–1571 (2021).

13) Akankwasa, G., Jianhua, L., Guixue, C., Changjuan, A. & Xiaosong, Q. Urine markers of podocyte dysfunction: A review of podocalyxin and nephrin in selected glomerular diseases. *Biomark. Med.* **12**, 927–935 (2018).

14) Furuta, I. *et al.* Association between nephrinuria, podocyturia, and proteinuria in women with pre-eclampsia. *J. Obstet. Gynaecol. Res.* **43**, 34–41 (2017).

15) Li, X. Z. & He, J. C. An update: the role of Nephrin inside and outside the kidney. *Sci. China Life Sci.* **58**, 649–657 (2015).

16) Kandasamy, Y., Smith, R., Lumbers, E. R. & Rudd, D. Nephrin - a biomarker of early glomerular injury. *Biomark. Res.* **2**, (2014).