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Analysis of Urinary Fibronectin Levels in Patients with Type 2 Diabetes Mellitus and Non-Diabetes Mellitus

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ABSTRACT

Diabetic nephropathy is a microvascular complication associated with high glucose levels in individuals with diabetes mellitus (DM). Fibronectin, an early marker of diabetic nephropathy, can indicate the occurrence and progression of renal damage. This cross-sectional study aimed to compare urinary fibronectin levels in patients with type 2 diabetes mellitus and non-diabetes mellitus. A sample of 50 DM patients was divided into two groups: the DM group and the non-DM group. Urinary fibronectin levels were measured using the ELISA method, while albuminuria levels were determined by the albumin-to-creatinine ratio (ACR). Statistical analysis was performed to assess the relationship between urinary fibronectin levels and urine albumin. Results: The average urinary fibronectin level in DM patients was 2.07 ± 3.04 ng/mL, which was slightly higher than the level observed in non-DM patients (1.09 ± 0.56 ng/mL). However, this difference was not statistically significant ($p > 0.05$). Additionally, there was no significant relationship found between urinary fibronectin levels and urine albumin ($p = 0.001$). Conclusion: The findings of this study indicate that urinary fibronectin levels in individuals with DM were slightly higher than those without DM. However, this difference did not reach statistical significance. The lack of a significant relationship between urinary fibronectin levels and urine albumin suggests that fibronectin may provide additional information about renal damage in DM patients, independent of albuminuria. Further research is necessary to explore the clinical significance of urinary fibronectin as a potential biomarker for diabetic nephropathy.

Keywords: Urinary fibronectin; Diabetes Mellitus; Urinary albumin; Diabetic nephropathy; Microvascular complications

INTRODUCTION

Diabetes Mellitus (DM) is a significant health concern worldwide, including in Indonesia. The prevalence of diabetes is increasing globally, and if left uncontrolled, it can lead to severe complications and even death. Elevated blood glucose levels characterize it due to abnormalities in insulin secretion, insulin action, or both. Insulin is a hormone the pancreas produces that helps regulate blood sugar levels. When the body cannot produce enough insulin or effectively use it, glucose builds up in the bloodstream, leading to hyperglycemia (Bonger et al., 2018). According to the *World Health Organization* (WHO) report on diabetes in 2019, the number of people affected by diabetes is projected to rise to 693 million by 2045 (Artasensi et al., 2020). This highlights the

growing burden of diabetes on global health. In the case of Indonesia, data from the Pusdatin Ministry of Health in 2018 estimated that there were 8.4 million people with diabetes in the country in 2000. By 2030, this number is expected to increase to 21.3 million, making Indonesia the fourth highest country in terms of the number of people with diabetes, following the United States.

The study conducted by Patten and Wang in 2021 found that plasma cellular fibronectin levels are elevated in diabetic patients. Specifically, they observed that diabetic subjects with cardiovascular risk factors had higher levels of plasma cellular fibronectin compared to those without risk factors (Patten & Wang, 2021). Additionally, the researchers performed univariate regression analysis and discovered that plasma cellular fibronectin levels were higher in individuals with type 2 DM than those with type 1 DM. This suggests that fibronectin levels may vary between different types of diabetes. Furthermore, the study indicated fibronectin is associated with diabetes complications in individuals with cardiovascular risk factors and those without such risk factors. This suggests that fibronectin may play a role in developing and progressing diabetes-related complications, regardless of cardiovascular risk factors.

Uncontrolled DM can cause various chronic complications, both microvascular and macrovascular (Brunton, 2016). Some diseases due to microvascular complications that can occur in patients with diabetes mellitus are diabetic retinopathy and nephropathy (Zhang et al., 2020). Diabetic nephropathy is a complication of DM in the kidneys which can end up in kidney failure. Kidney disease (nephropathy) is the leading cause of death and disability in people with diabetes mellitus (Abdelsalam et al., 2020; Yu et al., 2020). Diabetic nephropathy (DN) or better known as Diabetic Kidney Disease (PGD), is a chronic microvascular complication of diabetes that occurs most often and develops in as many as 15-25% of patients with type 1 diabetes mellitus (T1DM) and 30-40% in patients with type 1 diabetes mellitus (T2DM). Histopathologically, in Diabetic Kidney Diseases (DKD) there is a thickening of the glomerular basement membrane, hypertrophy, and mesangial expansion with accumulation of extracellular matrix proteins such as fibronectin, collagen, and laminin (Bandiara & Soelaeman, 2011).

Diabetic nephropathy is characterized by excessive matrix protein deposition which eventually causes end-stage renal failure (Buse et al., 2020; Davies et al., 2022; Dietlein, 2019). Matrix-triggering mediators that can increase protein deposition include transforming growth factor-β (TGF-β), which has been shown to increase serum transcription and Serine/threonine-threonine-protein kinase (SGK1)-induced glucocorticoid kinases (Zhang et al., 2018). The kinase was initially cloned as a glucocorticoid-inducible gene and was later shown to be strongly regulated by mineralocorticoids. SGK1 is expressed in fibrous tissues, such as diabetic nephropathy (Lin et al., 2014; Schena & Gesualdo, 2005).

The research on urinary fibronectin has not been widely carried out in Indonesia, including in Makassar, so researchers are interested in raising this title to know urinary fibronectin levels in people with type 2 DM.

METHODS

Location and Research Design

The study was conducted at the Clinical Pathology Laboratory, Hasanuddin University State Hospital Makassar, and the Hasanuddin University Medical Research Center (HUM-RC) Laboratory. This research method was carried out using observational analytic methods, and the approach used in this study used a cross-sectional study design. This study analyzed urinary fibronectin in type 2 DM patients to see the development of DM into diabetic nephropathy.

Population and Sample

The study population was adults with type 2 DM and non-diabetics. This study's sample was taken from adults with type 2 DM and non-diabetes mellitus who fit the inclusion

criteria. The sample size used in this study was calculated using the following formula:

$$\frac{n1 = n2 = [(Z\alpha + Z\beta)\, S\,]^2}{x1\text{-}x2}$$

Infomation :
n1= n2 : Minimum sample size
Zα : Derivate α = 5% = 1,645
Zβ : Devariate β = 20%
S : Standard intersection
X1-X2 : Minimum mean difference

$$n1 = n2 = \left[\ \frac{(1{,}645 + 0{,}842)\ 8}{4}\ \right]^2$$

$$= \left[\frac{2{,}487)\ 8}{4}\ \right]^2$$

$$= [19.896]^2$$

$$4$$

$$= 4{,}974^2$$

$$= 24{,}74 \rightarrow 25 \text{ sample}$$

Sampling Method

The sampling method in this study was non-probability purposive sampling, which is a sampling technique by selecting samples from among the population according to the researcher's wishes so that the sample can represent previously known population characteristics. Measurement of urinary fibronectin levels used the Elisa Ryder Enzyme-Linked Immunosorbent Assay (ELISA) method with an insert kit from the Assay Genie brand produced from Ireland, and measurement of urine albumin levels using the Cobas 311 immunoturbidimetric method. This research was conducted after ethical approval from the Health Research Ethics Commission Hasanuddin University Faculty of Medicine-UNHAS State Higher Education Hospital with ethical number 562/UN4.6.4.5.31/PP36/2022.

Data Analysis

Data processing was performed using the windows computer program SPSS (Statistical Package for the Social Sciences) version 23. The data analysis used was bivariate. The statistical test uses the Mann-Whitney test. Data not normally distributed using the Spearman Rank correlation test. The test results are significant if $p \leq 0.05$.

RESULT

Based on Table 1, it can be concluded that the total history of DM with T2DM criteria was 25 respondents (100%), non-DM 25 respondents (50%), criteria for male gender were 24 respondents (48%) and female 26 respondents (52%)), age criteria 30-39 years as many as 19 respondents (38%), 40-49 years as many as 6 respondents (12%), 50-59 years 16 respondents (32%), 60-69 years 8 respondents (16%), 70-79 years 1 respondent (2%). There were 38 respondents (76%) in the category of <30 mg/g urine albumin, 12 respondents (24%) in the category of ≥30 mg/g category.

Table 1. Subjects Characteristics

Characteristics	n	%
Gender		
Male	24	48
Female	26	52
Age (years)		
30 – 39	19	38
40 – 49	6	12
50 – 59	16	32
60 – 69	8	16
> 70	1	2
DM history		
DMT2	25	50
Non-DM	25	50
Subjects based on ACR (mg/g)		
< 30 (T2DM with DN)	38	76
≥ 30 (T2DM without DN)	12	24

Table 2. Comparison of Mean Urinary Fibronectin Levels in T2DM and Non-DM Subjects

Parameter	Characteristics	Mean	SD	p
Urinary Fibronectin (ng/mL)	T2DM	2.07	3.04	0.409
	Non-DM	1.09	0.56	
Urinary Albumin (mg/g)	T2DM	129.1	129.1	< 0.001
	Non-DM	4.88	4.88	
Urinary Fibronectin (ng/ml)	T2DM with DN	2.88	4.02	0.514
	T2DM without DN	1.33	1.52	
Urinary Albumin (mg/g)	T2DM with DN	258.37	320.78	< 0.001
	T2DM without DN	9.76	5.76	

**Mann-Whitney Test*

The urinary fibronectin value of respondents with T2DM and non-DM patients in Table 2, namely in the DMT2 group an average of 2.07 ng/mL higher than non-DM 1.09 ng/mL, but not statistically significant with $p = 0.409$ ($p > 0.05$).

The average urine albumin in the T2DM and non-DM groups in Table 2, namely in the DMT2 group the average urine ACR value was 129.1 ± 251.49 mg/g higher than that of non-DM 4.88 ± 3.16 mg/g but significantly different statistic with $p < 0.001$.

Table 2 shows the mean levels of fibronectin in the T2DM group with and without nephropathy. The group with nephropathy had an average level of 2.88 ± 4.02 higher than the average without nephropathy of 1.33 ± 1.52 ng/mL but was not statistically significantly different ($p=0.514$).

Based on Table 2, the average urine albumin value for each group of T2DM with nephropathy with an average ACR level of 258.37 ± 320.78 mg/g was higher than the average without nephropathy, which was 9.76 ± 5.76 mg/g with $p<0.001$.

Based on Table 3, the results of the correlation test between urine fibronectin and urine albumin levels in T2DM respondents obtained a correlation value (r) of 0.325 on the correlation scale this value indicates a weak relationship. The p value obtained was 0.113 which was greater than 0.05 indicating that there was no significant relationship between fibronectin and urine albumin levels in T2DM respondents.

Table 3. Correlation Test of Urinary Fibronectin Levels and ACR in T2DM Subjects

		Albumin/Creatinine Ratio (mg/g)
Urinary Fibronectin (ng/ml)	R	0.325
	P	0.113
	N	50

**Spearman-Rho Test*

Table 4 shows the results of the correlation test between urinary fibronectin and urine albumin levels in nephropathy DM obtained a correlation value (r) of 0.308 on a correlation scale at this value indicating a weak relationship. The p value obtained was 0.331 which was greater than 0.05 indicating that there was no significant relationship between urinary fibronectin and urinary albumin levels in nephropathy DM.

Table 4. Correlation Test of Urinary Fibronectin Levels and ACR in T2DM Subjects With DN

		Albumin/Creatinine Ratio (mg/g)
Urinary Fibronectin (ng/ml)	R	0.308
	P	0.331
	N	50

**Spearman-Rho Test*

Based on Table 5, it shows the results of the correlation test between urinary fibronectin and urine albumin levels in DM without nephropathy, a correlation value (r) of 0.496 on the correlation scale at this value indicates a weak relationship. The p value obtained was 0.085 which was greater than 0.05 indicating that there was no significant relationship between

urinary fibronectin and urine albumin levels in DM without nephropathy.

Table 5. Correlation Test of Urinary Fibronectin Levels and ACR in T2DM Subjects Without DN

		Albumin/Creatinine Ratio (mg/g)
Urinary Fibronectin (ng/ml)	R	0.496
	P	0.085
	N	50

**Spearman-Rho Test*

DISCUSSION

Type II diabetes mellitus is the most common type of DM and accounts for nearly 95% of all cases. Type II diabetes mellitus often goes undiagnosed for years because hyperglycemia develops gradually. Type II diabetes mellitus is caused by insulin resistance or tissue insensitivity to insulin. This study aims to analyze urinary fibronectin levels in patients with type 2 diabetes mellitus and non-diabetes mellitus. Sampling was carried out at Makassar University Hospital with a total sample of 50 subjects, which were divided into 2 groups, namely the diabetes mellitus and non-diabetes mellitus. Based on Table 1 female with age criteria 30-39 years has DM more than male because increased fat content in women is higher than men so that the factor of DM in women is 3-7 times higher than in men 2-3 times (Kautzky-Willer et al., 2016; Kendagor et al., 2018)

Based on the study results, Table 2 shows the results of a comparative test for urinary fibronectin levels in T2DM and non-DM. The mean value of urinary fibronectin in T2DM subjects (2.07 ng/mL) was higher than non-DM (1.09 ng/mL). This is because an increase in plasma levels of fibronectin indicates an injury to the wall and extracellular matrix. Increased fibronectin synthesis and decreased degradation of extracellular matrix components such as fibronectin are seen when blood glucose is high. This accumulation indicates damage to kidney function (Hasanah, 2014).

Table 3 shows statistically significant differences in urine albumin levels in T2DM and non-DM subjects with a p-value <0.001 <0.05 with an average urine albumin level in T2DM subjects 129.1 mg/g higher than urine albumin levels in non-DM subjects (4.88 mg/g). This is in line with the study of (Maciorkowska et al., 2019) which stated that diabetic subjects with cardiovascular risk factors had higher plasma cellular fibronectin levels than those without risk factors.

Table 4 shows statistically significant differences in urinary albumin levels in nephropathy and without nephropathy in T2DM and non-DM subjects, where the mean value of each urinary albumin group with nephropathy average ACR level of 258.37 ± 320.78 mg/g higher than the average without nephropathy, namely 9.76 ± 5.76 mg/g with p<0.001. This is because, in T2DM subjects, there is an increase in blood glucose which causes dysfunction of the mesangial matrix and GBM. The accumulation of ECM components experiences an imbalance between synthesis and degradation of ECM components so that it will result in various kidney diseases it can increase urine albumin (Gunasekara et al., 2020; Mizdrak et al., 2022; Xiao et al., 2022). Diabetic nephropathy is characterized by excessive extracellular matrix deposition in the kidney, causing glomerular mesangial expansion and fibrosis (Jana S, Mitra P & Roy S, 2022). Previous studies have linked diabetic nephropathy with hyperglycemia-induced extracellular matrix deposition and mesangial cell dysfunction leading to increased urinary albumin (Karasawa et al., 2020; Trimarchi & Coppo, 2019). Urinary albumin is one of the first proteins to be detected in the urine in case of kidney damage. Increased albumin in the urine indicates the severity of impaired kidney function (Chen et al., 2022; Moh et al., 2023; Moulton et al., 2023).

Table 5 shows a statistically significant difference in urinary fibronectin levels in nephropathy and without nephropathy in T2DM and non-DM subjects. mL but not statistically significantly different (p=0.514). In line with previous studies, (Huhn et al., 2016; Mohieldein

et al., 2007) found no significant difference in fibronectin levels between diabetic patients as a whole and the non-DM control group, which suggests that fibronectin does not play a major role in diabetic nephropathy but does not rule out the possibility that there are abnormalities on fibronectin tissue components in diabetes.

Table 3 shows that there is no significant relationship between urinary fibronectin and urinary albumin levels, where the results of the correlation test between urinary fibronectin and urinary albumin levels obtained a correlation value (r) of 0.325 on a correlation scale this value indicates a weak relationship. The p-value obtained was 0.113, which was greater than 0.05, while Table 4 shows that there was no significant relationship between urinary fibronectin levels and urinary albumin in nephropathy DM where the results of the correlation test between urinary fibronectin levels and urine albumin in nephropathy DM obtained the value correlation (r) of 0.308 in the correlation scale at this value indicates a weak relationship. The p-value obtained is 0.331, which is greater than 0.05. This aligns with the finding that urinary albumin in diabetes leads to glomerular basement membrane thickening and increased mesangial volume, largely due to increased mesangial matrix (Slate-Romano et al., 2022).

In the diabetic kidney, there is an increase in fibronectin in the mesangial matrix and also in the capillary walls (Belly et al., 2020; Kanta et al., 2022). Because of this accumulation of fibronectin in the mesangium in diabetes, fibronectin is a major component of plasma and basement membranes. In addition, information exists that high glucose can induce overexpression of fibronectin (Dhyani et al., 2016; Fox et al., 2011; Klemis et al., 2017). Table 5 shows that there is no significant relationship between urinary fibronectin levels and urinary albumin in DM without nephropathy, where the results of the correlation test between urinary fibronectin levels and urinary albumin in DM without nephropathy obtained a correlation value (r) of 0.496 on a correlation scale at this value indicating weak relationship. The p-value obtained is 0.085, which is greater than 0.05. Previous studies reported that circulating cellular fibronectin was increased in diabetic patients with macroalbuminuria compared to diabetic patients with normoalbuminuric and microalbuminuria (Perakakis et al., 2017; Yano et al., 2021). It was also reported that higher urinary albumin excretion was independently associated with increased circulating cellular fibronectin in diabetes (Ballana et al., 2011; C. Lin et al., 2021). According to this study, fibronectin is associated with diabetes complications with cardiovascular risk factors and those with no risk factors (Komala et al., 2016; Li et al., 2020; Zheng et al., 2019).

CONCLUSIONS

There was no significant difference between urinary fibronectin in T2DM and non-DM conditions, nor was there a significant correlation between urinary fibronectin and urinary albumin levels.

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