

Analysis of Urine Type IV Collagen in Diabetic Nephropathy Patients

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Abstract

Type 2 diabetes mellitus is a disease characterized by hyperglycemia (increased blood sugar levels). Hyperglycemia from diabetes mellitus affects many organs of the body, causing complications, one of which is diabetic nephropathy. Type IV collagen is a major component of the glomerular basement membrane (GBM) and is mesangial, the level of type IV collagen in a urine sample reflects the rate of matrix turn over in the injured kidney. Urinary Type IV collagen recently emerged as a marker for the early detection of diabetic nephropathy. This research aimed to measure and to compare urine type IV collagen levels in patients between non diabetic nephropathy and diabetic nephropathy. The research method used was the observational analytic with cross sectional design. The data in this research used 50 samples, of which 25 samples were from diabetic nephropathy and 25 samples were from non diabetic nephropathy. Type IV collagen levels were examined using the ELISA method. The results showed that the results of the statistical analysis of urine type IV collagen comparison test in nephropathy and non nephropathy is 26.28 ng/mL and 12.50 ng/mL. Correlation test results of urinary type IV collagen levels ACR urine is 0.536 ($p \leq 0.05$). The conclusion is that there are differences in urinary type IV Collagen in nephropathy and non nephropathy patients, next there is a correlation between urinary ACR and urinary type IV collagen in diabetic nephropathy.

Keywords: Type 2 Diabetes Mellitus, Type IV Collagen, Diabetic Nephropathy

1. Introduction

Type 2 diabetes mellitus according to the American Diabetes Association (ADA) is a group of metabolic diseases with hyperglycemia characteristics that occur due to abnormalities in insulin secretion, insulin action or both. Diagnostic criteria for type 2 DM are the results of blood glucose examination when ≥ 200 mg/dL, fasting blood glucose ≥ 126 mg/dL, OGTT ≥ 250 mg/dL, and/or HbA1c $> 6.5\%$. (Fitriyani, 2012). Diabetes Mellitus can cause various complications, including diabetic retinopathy, diabetic nephropathy and diabetic neuropathy. According to the Basic Health Research Results (Riskesdes) report in 2018, it shows that the prevalence of DM is obtained based on the results of blood sugar checks on residents aged 15 years and over by 2.0%.

Diabetes Mellitus (DM) experiences an increasing every year and becomes a threat to world health. The prevalence of type 2 DM accounts for 90% of all diabetes and is one of the most common diseases in the world (International Diabetes Federation, 2019). The development of the prevalence of diabetes mellitus in Indonesia is increasing. In 2010 it was 2.9% and CFR 3% with a total of 7 million cases and

210,000 deaths. Prevalence increased in 2013 by 6% and CFR 6% with a total of 14 million cases and 840,000 deaths. Indonesia is ranked 10th with the most DM deaths in the world (WHO, 2014).

In DM conditions, hyperglycemia causes thickening of the glomerular basement membrane and mesangial expansion due to accumulation of extracellular matrix (ECM) proteins such as Type IV Collagen. Thickening of the glomerular basement membrane and mesangial expansion are hallmarks of the morphological changes observed in diabetic nephropathy. One of the markers for diagnosing the early stages of diabetic nephropathy is Urine Type IV Collagen. Several studies have shown that urinary type IV collagen (u-CIV) levels increase gradually with the development of diabetic nephropathy. It appears that u-CIV represents a reliable index for the onset and progression of diabetic nephropathy. A prospective study in type 2 DM patients found that urinary type IV collagen was found to be more sensitive than albuminuria for detecting renal impairment.

Recently the extensive search for better indicators of diabetic nephropathy has yielded at least one promising candidate, one of which is collagen type IV. Clinical trials have shown increased urinary

collagen type IV levels in diabetic patients compared to healthy controls and in patients with primary glomerular disease (Kikkawa R, et al. 1997). Furthermore, urinary type IV collagen levels increase gradually as diabetic nephropathy progresses. It appears that urinary collagen type IV may represent a reliable index for the onset and development of diabetic nephropathy (Watanabe H, et al. 2000).

2. Materials And Methods

Location and Research Design

Sampling was carried out at the Clinical Pathology Laboratory, Hasanuddin University State University Hospital. Furthermore, the research was carried out at the Hasanuddin University Medical Research Center (HUM-RC) Laboratory, State University Hospital, Hasanuddin University, Makassar. The method of this research was carried out using observational analytic methods with a cross-sectional research design.

Population and Sample

The target population of this research was a population of adults with type 2 diabetes mellitus and non-diabetes mellitus. The sample size used in this research was calculated using the following formula:

$$n1 = n2 = [(Z\alpha + Z\beta) S]^2$$

x1-x2

information:

n1 : minimum size sample DM type 2

n2 : minimum large Non-DM sample

Z α : dalpha raw erivate, calculated from type I error (Z α value = 1.645)

Z β : dbeta raw erivate, calculated from type II error (Z β value = 0.845)

S: the intersection obtained (Value S = 8)

X1-X2 : the minimum mean difference that is considered meaningful (Value X1-X2 = 4)

$$n1 = n2 = [(1,645 + 0,842) 8]^2$$

4

$$= [(2,487) 8]^2$$

4

$$= 4,9742$$

$$= 24,74 \text{ roundedan to } 25.$$

Based on calculations using the above formula, a minimum sample of 25 samples for the type 2 DM group and 25 samples for the non-diabetic group with a total of 50 people is obtained.

Sampling Method

The sampling method in this research was non probability purposive sampling, namely determining the sample by selecting a sample among the population according to the wishes of the researcher, so that the sample can represent the characteristics of the population that have been known before. This research was conducted after obtaining ethical approval from Health Research Ethics Commission (KEPK) Faculty of Medicine Hasanuddin University UNHAS State University

Hospital (RSPTN UH) with ethical number 562/UN4.6.4.5.31/PP36/2022.

Data Analysis

Data processing was carried out using the Windows SPSS computer program. Data analysis used is bivariate analysis. The Statistical test uses the Independent T test or the Mann-Whitney test. If normally distributed using the Pearson correlation test and if not normally distributed using the Spearman Rank correlation test. Th test result significant if ≤ 0.05 .

3. Results and Discussion

Table 1. Characteristics of the Research Subject

Characteristic	n	%
Gender		
Men	24	48,0
Woman	26	52,0
Age		
30 – 40 years old	5	10,0
41 – 50 years old	6	12,0
51 – 60 years old	16	32,0
61 – 70 years old	19	38,0
> 70 years	4	8,0
Albuminuria Levels		
< 30 mg/g	25	50,0
> 30 mg/g	25	50,0
Source: Primary Data		

Table 2. Comparison of Collagen Type IV Urin Levels in Nephropathy and Non Nephropathy

	Condition	Mean	Std. Deviation	p-value
Collagen Type IV Urine	Nephropathy	26.28	11.29	<0.001
	Non Nephropathy	12.50	9.25	

Table 3. Relationship of Urinary Type IV Collagen Levels with Urine ACR

Relationship	Correlation Value	p-value
Collagen Type IV Urine and Urine ACR	0.536	<0.001

Table 4. AUC, Sensitivity, Specificity, Cut-off point Collagen type IV urine

Variable	AUC (95% CI)	Sensitivity	Specificity	Cut - off point
Collagen Tipe IV urine	0.850 (0.739 - 0.961)	0.800	0.800	17.24

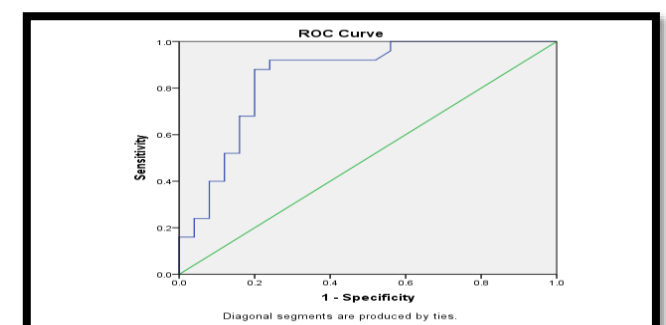


Figure 1. ROC Curve Collagen type IV urine results

Table 1 shows the characteristics of the research subjects based on male sex were at 24 respondents (48.0%) and based on women sex were at 26 respondents (52.0%), based on the age of the research subjects, the most number of age is the age of 61-70 years with 19 respondents (38.0%), age 30-40 years at 5 respondents (10.0%), age 41-50 years at 6 respondents (12.0%), age 51-60 years at 16 respondents (38.0%), and the least number of age is >70 years with the number of 4 respondents (8.0%), based on urinary ACR <30 mg/g of 25 respondents (50.0%) with the same number of urinary ACR \geq 30 mg/g of 25 respondents (50.0%).

Table 3 shows that the average urine type IV collagen in nephropathy was 26.28 with a standard deviation of 11.29, while the average urine type IV collagen in non-nephropathy was 12.50 with a standard deviation of 9.25. This shows that the average urinary type IV collagen in nephropathy conditions is greater than urinary type IV collagen in non-nephropathy conditions. The results of the comparison test show a p-value of 0.001 which is smaller than 0.05 (p-value <0.05). This shows that there is a significant difference between urinary type IV collagen in nephropathy conditions and urinary type IV collagen in non-nephropathy conditions.

Table 4 shows the results of the correlation test between urinary type IV collagen and albuminuria levels using the Spearman rank correlation test. A correlation value (r) of 0.536 is obtained; on a correlation scale this value indicates a moderate correlation. The p-value obtained was 0.000 which was smaller than 0.05 (p-value <0.05) indicating that there was a significant relationship between urinary type IV collagen and urinary ACR.

Based on Figure 1, it shows that the prevalence of nephropathy cases is 50%. At the output, there is a ROC curve which shows that the Collagen Type IV Urine value has a good diagnostic value because the curve is far from the 50% line and close to 100%. The AUC value obtained from the ROC method is 85%. Statistically the AUC value of 85% is classified as strong. The AUC value of 85% means that if the Collagen Type IV Urine value is used to diagnose the presence or absence of DM sufferers with nephropathy in 100 patients, then the right conclusions will be obtained in 85 patients. Based on the confidence interval, we know that the AUC value of Collagen Type IV Urine in the population ranges was from 73.9% - 96.1%.

Based on AUC, Sensitivity, Specificity, Urine Type IV Collagen Cut-off point (Table 4) shows a value of \geq 17.245 with a sensitivity value of 80% and a specificity of 80%. This means that patients who have Collagen Type IV Urine \geq 17.245 will be diagnosed as patients with nephropathy. The statistically recommended cut point is \geq 17.245. The sensitivity and specificity values of the cut point \geq 17.245 are 80% and 80 respectively %.

4. Discussion

The purpose of this research was to determine and

to compare urinary type IV collagen levels in nephropathy and non-nephropathy patients. The results of this research indicated that urinary type IV collagen levels in nephropathy patients were higher than urinary type IV collagen levels in non-nephropathy patients (Table 3). The results of the comparison test showed a p-value of 0.001 which was smaller than 0.05 (p-value <0.05). This result showed that there was a significant difference between urinary type IV collagen in nephropathy conditions and urinary type IV collagen in non-nephropathy conditions. These results were in line with previous studies which reported that urinary type IV collagen excretion increased in patients with diabetic nephropathy (Yagame et al. 1997; Iijima et al. 1998).

The results of this research were based on a correlation test of urinary type IV collagen levels with albuminuria levels showing a p-value obtained of <0.001 which is smaller than 0.05 (p-value <0.05) indicating that there is a significant relationship between urinary collagen type IV and Urinary ACR. This finding is in line with the findings' Pavai Sthaneshwar & Siew-Pheng Chang. Their research showed that urinary type IV collagen excretion was increased in diabetic patients with microalbuminuria and there was a significant correlation between albumin excretion and urinary collagen type IV excretion (p <0.0001). The other research by Kikkawa et al has reported the similar observations. Their research noted that the excretion of type IV collagen increased in accordance with the increase in urinary albumin excretion.

The results of Watanabe's research reported that urinary excretion of type IV collagen was higher in diabetics with microalbuminuria and clinical proteinuria than those with normoalbuminuria. Iijima's research showed that microalbuminuria was present in 25% of diabetic patients with higher urinary levels of type IV collagen after 2 years of follow-up. However, Ishimitsu also reported that urinary collagen type IV was significantly correlated with systolic pressure and age. Therefore, there is still doubt about the specificity of urinary type IV collagen as an indicator of diabetic nephropathy. Research by Balu Mahendran, et al. Based on the results of the study, multiple regression analysis showed that plasma and urine type IV collagen levels were significantly correlated with albumin creatinin ratio (ACR) in micro-diabetic and macroalbuminuria patients. This implies that plasma and urine type IV collagen may be useful as early diagnostic markers to detect nephropathy even before the onset of microalbuminuria.

In Case Processing Summary (Table 4) we got information that nephropathy subjects were 25 out of 50 subjects. Thus the prevalence of nephropathy cases is 50%. At the output, there is a ROC curve which shows that the Collagen Type IV Urine value has a good diagnostic value because the curve is far from the 50% line and close to 100%. The AUC value obtained from the ROC method is 85% (95%IK73.9%

- 96.1%), $p < 0.001$. statistically the AUC value of 85% is classified as strong. The AUC value of 85% means that if the Collagen Type IV Urine value is used to diagnose the presence or absence of DM sufferers with nephropathy in 100 patients, then the right conclusions will be obtained in 85 patients. Based on the confidence interval, we know that the AUC value of Collagen Type IV Urine in the population ranges was from 73.9% - 96.1%.

The hypothesis test was carried out to compare the AUC obtained and the AUC result was compared to the AUC value of 50%. The value of $p < 0.001$ ($p < 0.05$) means that the AUC Collagen Type IV Urine value is significantly different from the AUC value of 50%. Clinically, the AUC Collagen Type IV Urine value is very satisfactory because it is greater than the expected minimum AUC value of 70%.

Based on AUC, Sensitivity, Specificity, Urine Type IV Collagen Cut-off point (Table 4) shows a value of ≥ 17.245 with a sensitivity value of 80% and a specificity of 80%. This means that patients who have Collagen Type IV Urine ≥ 17.245 will be diagnosed as patients with nephropathy. The statistically recommended cut point is ≥ 17.245 . The sensitivity and specificity values of the cut point ≥ 17.245 are 80% and 80 respectively%.

5. Conclusions and Recommendations

The results of a comparative test of type IV collagen in urine in nephropathy and non-nephropathy patients showed that the results of Urinary type IV collagen levels in nephropathy patients were higher than urinary type IV collagen levels in non-nephropathy patients. This shows that there is a significant difference between urinary type IV collagen in nephropathy conditions and urinary type IV collagen in non-nephropathy conditions. In future studies, it is proposed to measure urinary collagen type IV levels in patients with advanced diabetic nephropathy who already require hemodialysis.

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